The diet, energetics and distribution of the freshwater crayfish *Paranephrops zealandicus* (White), in Lake Georgina, South Island, New Zealand.

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Paranephrops zealandicus (White, 1847)
(Right: female, carapace length 38 mm)

In New Zealand our freshwater crayfish
belong to the genus Paranephrops,
so called from its external resemblance
to the Norwegian saltwater
lobster, (Nephrops) . . . Chilton (1913).

♀

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ABSTRACT

The diet and the temporal and spatial distribution of the freshwater crayfish Paranephrops zealandicus White were investigated in Lake Georgina, Canterbury. The digestive ability and efficiency of the crayfish were also examined in the laboratory.

The diet of the crayfish during the period of the field study (January 1986 to November 1986) consisted largely of macrophyte detritus (principally Elodea canadensis), epilithic algae and exoskeletal material. Fish and other animal tissues appeared rarely in crayfish guts.

Crayfish activity was investigated once every two months using a trapping programme. Activity was high in May and September and low in July. The September increase coincided with increases in moult frequency and egg production.

Digestive enzyme activity was investigated using standard assay techniques. Hepatopancreas extracts containing digestive enzymes showed catalytic activity toward nine selected substrates. Microbial activity was implicated in the breakdown of three substrates, microcrystalline cellulose, laminarin and collagen.

Ingestion rate and assimilation efficiency were affected by environmental temperature. At 15^{o}C the rate of ingestion of fresh and decaying Elodea canadensis was

highly variable and assimilation efficiency averaged 21 %. At 5°C, feeding rate was greatly reduced but mean assimilation efficiency was 87 %. Digestive enzyme activity also increased at low temperatures and may compensate for reduced ingestion to some extent. Crayfish size influenced ingestion rate but not assimilation efficiency. Sex and degree of decomposition of Elodea did not affect assimilation efficiency or ingestion.

Temperature and photoperiod appear to have a strong influence on the crayfish population of Lake Georgina. They affected epilithic algal production and therefore provision of an important source of food, crayfish distribution, and both physical (i.e. ingestion and winter activity decrease) and metabolic (i.e. enzymic catalysis and assimilation efficiency) activity.

CHAPTER 1

GENERAL INTRODUCTION

GENERAL INTRODUCTION

1.1 Introduction

At present two species of freshwater crayfish (Paranephrops zealandicus and P. planifrons) are recognised in New Zealand. P. zealandicus is found on the eastern side of the South Island from North Canterbury to Southland and Stewart Island, whereas P.planifrons is distributed throughout the North Island, in Marlborough and on the west of the South Island. There are indications that this separation was relatively recent geologically speaking, possibly as little as 5.2 million years ago (Hopkins, 1970), since the the geographical barrier between them, the Southern Alps, is thought to have reached what is essentially its present configuration at the beginning of the Pliocene (Fleming, 1962). Speciation also may be incomplete as successful cross-breeding has been carried out producing fertile offspring (A. Devcich pers comm 1985). For this reason, the two forms may be better designated as subspecies than species.

In New Zealand, freshwater crayfish are found in a wide variety of habitats from sea level to about 2000 m asl (Carpenter, 1977). Habitats may have mud or gravel substrates and include ponds, lakes and streams of various sizes. Crayfish make burrows

where the substrate is suitable, especially where the current is swift (Hopkins 1970). Feeding takes place mainly at night, and most crayfish are considered to be omnivorous bottom feeders (Devcich 1979) which consume a wide variety of autochthonous and allochthonous detritus. Less decomposed vegetation and living aquatic macrophytes are also eaten by P. planifrons (Devcich 1979).

In this study I examined the diet of a lake population of P. zealandicus from January 1986 to November 1986 and investigated the enzyme reservoir and assimilation efficiencies of the crayfish in laboratory studies. Fluctuations in distribution of crayfish, within the study site (Lake Georgina) over the same period and in relation to food availability, light and temperature were also considered. Chapter 1 of the thesis summarises relavent New Zealand research into the genus and describes the study site. Succeeding chapters commence with synopses of local and overseas studies covering the subject area with which each chapter is concerned. Chapter 2 describes and discusses the diet and distribution of the population in Lake Georgina. Chapter 3 describes the enzyme reservoir. Chapter 4 investigates assimilation efficiencies and food ingestion rates in relation to temperature, size, sex and food condition. Chapter 5 is a summary and synthesis of the previous chapters and concludes the study.

1.2 Study Site

Lake Georgina (43°18'S 171°34'E), is a small mesotrophic (Spencer 1978) kettlehole lake in the Rakaia River catchment, 2 km

ESE of Lake Coleridge in the Southern Alps (**Fig 1.1**). It is situated on glacial moraine debris at an altitude of 540m a.s.l. and is surrounded by tussock and grazed, high country pasture. The lake has an area of about 20 ha (0.75 * 0.3 km) and a maximum depth of 12 m (Stout, 1969; Livingston, Biggs and Gifford, 1986; Glenny et al.,
1987).

Water movement into the lake is primarily through the surrounding glacial gravels and inflow is most apparent from a spring situated in the deepest part of the lake (**Fig 1.2**). A small permanent stream at the southern end of the lake provides the only surface inflow. Surface outflow is from an ephemeral branch of the Scamander Stream (**Fig 1.1**). During the period of the study (December 1985 - January 1987), flow was observed only from September to December 1986.

Three major vegetation zones occur within the lake (**Fig 1.3**). In the shallows, epilithon covers greywacke cobbles and boulders extensively and consists of a dense matrix of filamentous algae (green, blue-green and diatoms), other diatoms, amorphous organic material and sediment. The quillwort Isoetes alpinus is the most conspicuous plant, and is found usually in a turf with diatoms and filamentous algae. Also scattered within this zone are patches of Myriophyllum sp., and Chara sp. (stonewort). The distribution of the three latter species varies with substrate size. Isoetes alpinus and Myriophyllum sp are usually associated with cobbles and Chara sp. occupies finer substrates (stones and pebbles) which form the lower depth limit of this zone and grade into the fine silt of the characean meadows.

Fig 1.1 Lake Georgina : Location in relation to surrounding waterways. Based on Fig. 3, Glenny (et al., 1987 - material used with permission) and NZMS 1 S65, S66, S73 and S74.

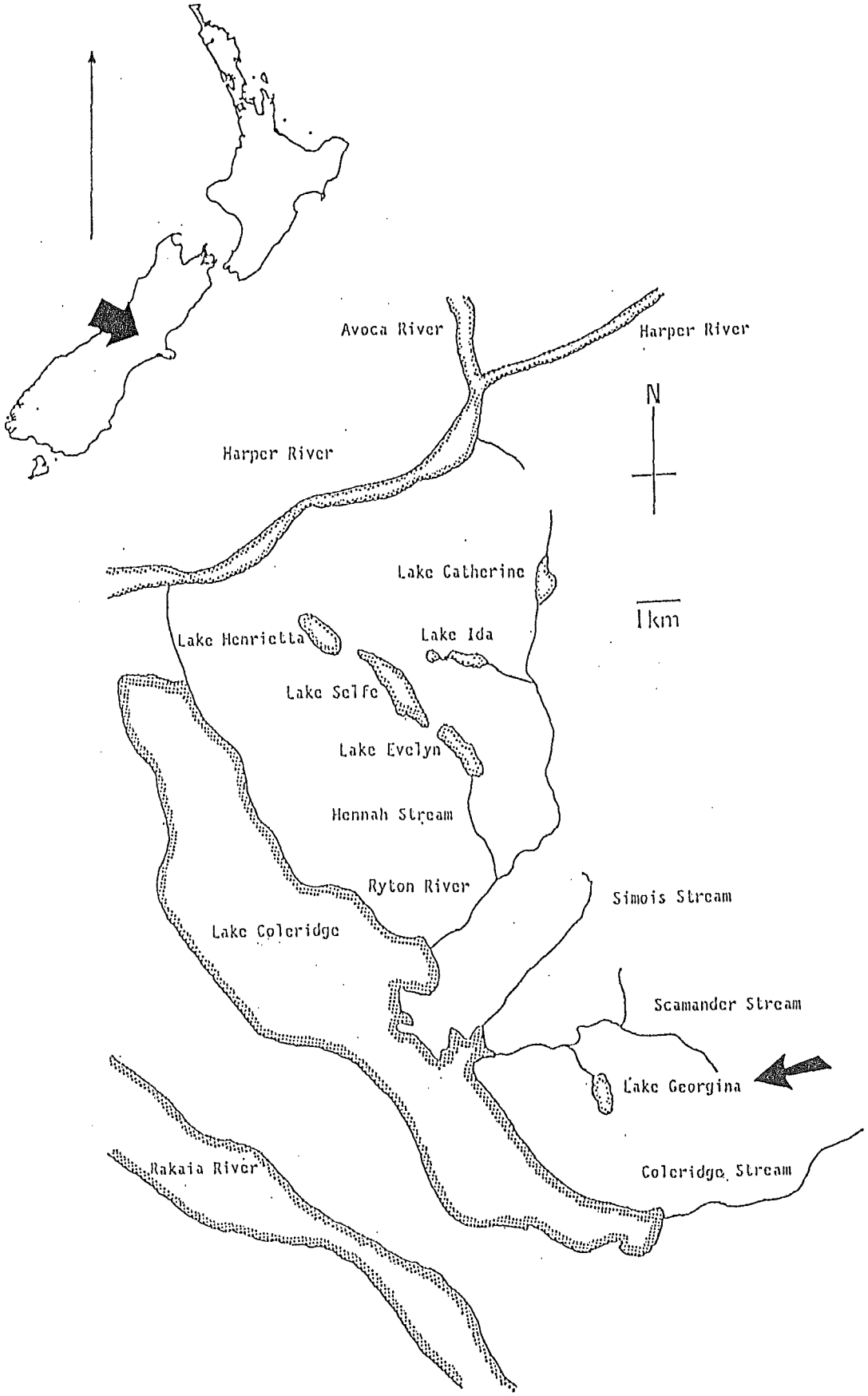

Avoca River
Harper River
Harper River
N
Lake Catherine
1km
Lake Henrietta
Lake Ida
Lake Selfe
Lake Evelyn
Hennah Stream
Ryton River
Simois Stream
Lake Coleridge
Scamander Stream
Lake Georgina
Rakaia River
Coleridge Stream

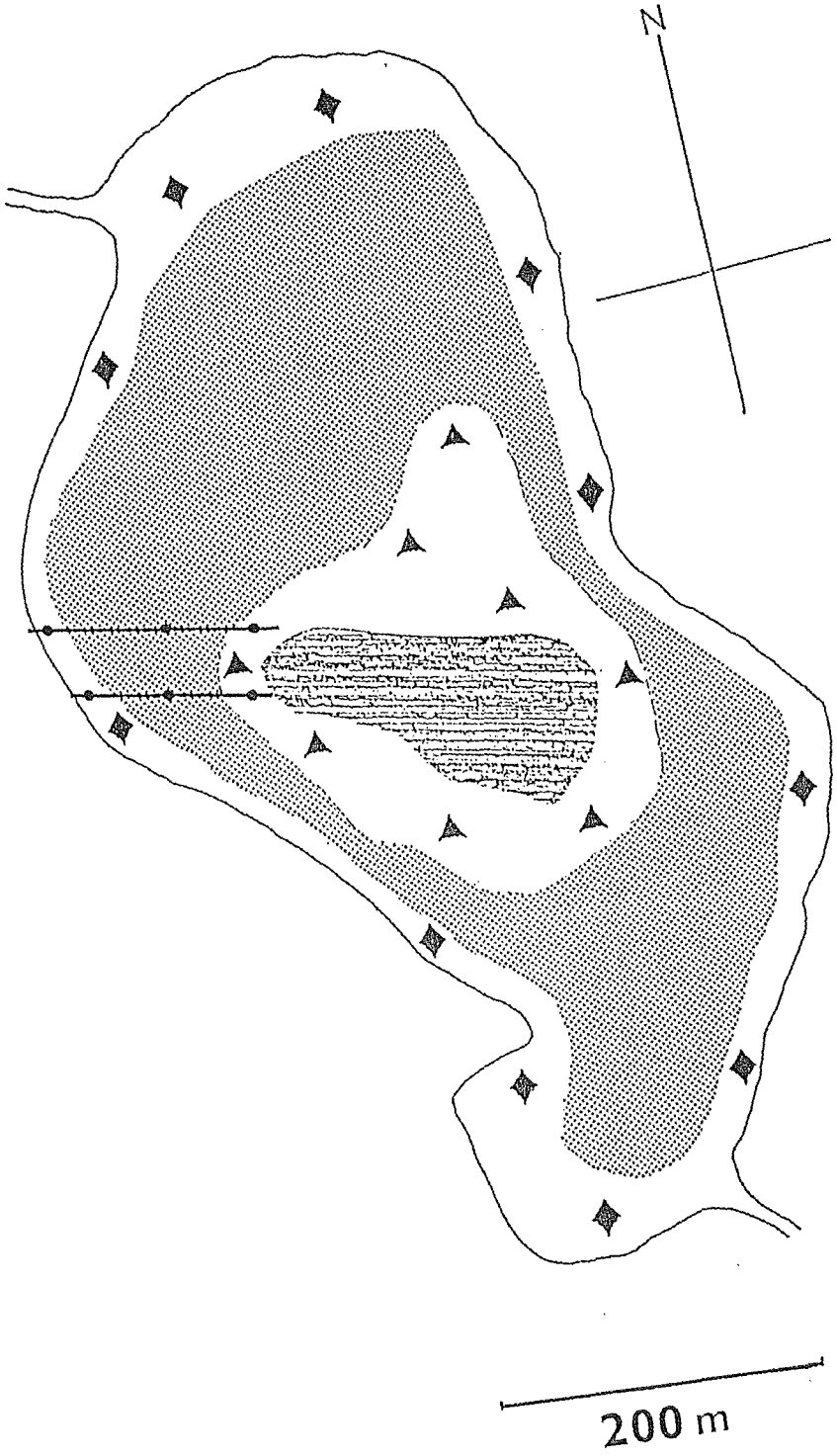
Fig 1.2 Lake Georgina : Bathymetric contours and position of transects used in diet and distribution study (Chapter 2). W - west, E - east. Contours based on own observations, and unpublished data (used with permission - V.M. Stout, 1987). Filled circles on transect lines show approximate sampling sites for crayfish and plant material.


N
1
2
3
4
6
8
10
12
W
E
200 m

Fig 1.3 Lake Georgina : Main vegetation zones and transects used to determine diel activity patterns (Chapter 2). Zones based on own observations and unpublished data (used with permission - V.M. Stout, 1987). Filled circles on transect lines show approximate sampling sites for crayfish.

Key:  Cobbles and turf;  Chara sp

 Elodea canadensis;  Fine Sediment.


N
200 m

The eastern lake boundary is formed by a series of lateral moraines which give the shallows on that side of the lake a steep slope (about 1:2). Boulders (> 256 mm) and large cobbles (128-256 mm) predominate. There is little turf or space for the attachment of macrophytes and the predominant community is epilithic. In contrast, the western boundary is a large shingle fan and the northern and southern shores are glacial debris (not moraine). All three of these shores have relatively gentle slopes (about 1:3.5) and the substratum is a pavement of large and small (16-32 mm) cobbles with occasional small boulders and areas of turf-Isoetes, Chara and Myriophyllum.

Below about 1 m depth, epilithon gives way to extensive charophyte beds (Chara spp.) which, in turn, are replaced by the Canadian pond weed, Elodea canadensis at a depth of about 2.5 m. The Elodea beds form an open canopy reaching a height of about 2m below which a loose 'subcanopy' of dead and decaying Elodea stems reaches 0.3 m in height. Chara, on the other hand, forms meadows of tightly packed plants 15-30 cm tall, and covers all of the available bottom substrate within its zone.

The vegetation stops at the lip of the spring (4.5-5.5 m depth). Below this level unstable fine sediments occur through which percolate the waters of the spring. The lake rarely develops a marked thermocline (V.M. Stout pers. comm.) presumably because of its shallow profile, deep water spring inflow and persistent wind (the valley is oriented in the direction of the prevailing winds, NW - SE).

The invertebrate fauna of the lake is depauperate relative to that of other lakes in the area (Glenny et al., 1987). Lake

Georgina has 0.5 to 3 orders of magnitude less invertebrate fauna, excluding crayfish, in terms of biomass and numbers than nearby Lakes Catherine, Evelyn, Selfe and Ida (**Fig 1.1**), but is the only lake containing Paranephrops zealandicus. The crayfish were introduced to the lake prior to 1940 (W.C. Clark, pers comm).

Brown trout (Salmo trutta), rainbow trout (Salmo gairdneri) and the upland bully, Gobiomorphus breviceps are present in the lake and regular introductions of trout are made by the local Acclimatisation Society. Bullies are very common, and often they are visible among rocks in the shallows. A pair of crested grebes (Podiceps australis) were resident on the lake during my study and visiting birds include Southern black-backed gulls (Larus dominicanus), flocks of paradise shelducks (Tadorna variegata) and Canada geese (Branta canadensis).

CHAPTER 2

DIET AND DISTRIBUTION

DIET AND DISTRIBUTION

2.1 Introduction

Crayfish are highly mobile, opportunistic omnivores (Momot et al., 1978; Capelli, 1985) which feed and therefore obtain energy at many trophic levels. The success of this polytrophic strategy may contribute to the general longevity of crayfish and allow continued growth and reproductive success despite seasonal or year-to-year fluctuations in particular food species. Estimates of crayfish lifespans range from 2 years for Orconectes clypeatus (Smith, 1953) to 8 years for Pacifastacus leniusculus (Flint, 1975) and possibly 20 years for Paranephrops planifrons (Devcich 1979).

The ability to feed at a variety of trophic levels is primarily a result of appendage and mouthpart stucture. The combination of chelae, chelate first and second walking legs, mandibles and gastric mill form an efficient food handling and maceration system and enable crayfish to break down most plant tissue (Gaeveskaya, 1966).

Although generally opportunistic, food preference by crayfish has been reported and may vary with species, age and sex. Fourth instar young-of-the-year of Orconectes immunis, O. propinquus and Cambarus robustus are described as obligate filter-feeders by Budd et al. (1977,1979), and juvenile Orconectes virilis (Momot et al.,

1978) and Astacus astacus (Jarvekugl, 1958 cited in Gaeveskaya, 1966) may feed more extensively on animal tissue than do adults of the same species. However, some adult crayfish can increase animal tissue consumption periodically in response to the higher energy demands associated with moulting (Jarvekulg, 1958) or gonad development (Gaeveskaya, 1966).

Spatial and temporal distribution of crayfish populations is affected by temperature and light, acting singly and interactively. Negatively phototactic behaviour (Quilter, 1975) and decreasing winter temperatures may produce direct effects such as nocturnalism and torpor, respectively, whereas photoperiod and temperature can indirectly influence food availabilty and foraging behaviour (Flint 1977). Such effects can be expressed by some component of a population (i.e. juveniles) or by the population as a whole.

Low winter temperatures affect activity levels directly by slowing metabolism until torpor is induced (Somers and Stechey, 1986; Quilter, 1975). Quilter (1975) reported that stream-dwelling P. zealandicus retreated to 'hibernacula' - hollows excavated under stones, during winter. Sometimes crayfish were found thickly covered with mud, debris and dead leaves, indicating extended periods of immobility. Similarly, Tack (1941), Momot (1967), Aiken (1968), Williams (et al., 1974), and Flint (1977) reported winter torpor and similar sheltering behaviour under stones and within burrows by other crayfish species. Seeking shelter during winter may decrease chances of predation, which otherwise might be high because of low activity levels. Quilter (1975) suggested that increased winter stream

flows near Dunedin probably encouraged shelter-seeking by P. zealandicus to avoid being swept downstream.

Lake populations of crayfish are affected by low winter temperatures (Somers and Stechey, 1986) and their reactions may be dependent on species and size-specific temperature tolerance. Thus, Somers and Stechey (1986) found that the activity of the Northern Hemisphere cambarid Cambarus clarkii was limited by low temperatures to a greater extent at the northern limits of its range than was the more northerly distributed Orconectes virilis. They also reported an increase in length of crayfish with decreasing temperature. Winter migration to deeper areas has been reported also, in a population of the astacid Pacifastacus leniusculus, in response to increased wave action (Flint, 1977) and to facilitate gonad maturation in Orconectes virilis (Aiken, 1968). Devcich (1979) suggested that winter migration to deeper water by Paranephrops planifrons in Lake Rotoiti (North Island) was a predator-avoidance response and he reported a reverse summer migration to the littoral as a result of of hypolimnetic deoxygenation.

Distribution of crayfish is affected also by seasonal food availability. Migration to the littoral during summer enables crayfish to make use of seasonally abundant living plant tissue and detritus, and enables energy requirements associated with an increased metabolic rate and gonadial development to be satisfied (Devcich 1979).

Many crayfish are nocturnal feeders (Penn, 1943; Devcich, 1979; Quilter, 1975; Abrahamsson, 1983) and their diel distributions are affected by interactions between light, shelter

and food proximity. The evolution of nocturnal feeding habits in the three families of freshwater crayfish (Cambaridae, Astacidae, and Parastacidae) may have been associated with predation pressure from day-active predators such as trout, perch and shags (Scott and Duncan 1967, Devcich 1979).

P. planifrons is negatively phototactic, and in Lake Rotoiti Devcich (1979) found a pattern of diel migration with shallow feeding areas occupied during the night and shelters (i.e. burrows, crevices, the aphotic zone) during the day. Similar light-mediated behaviour was reported by Quilter (1975) for P. zealandicus in streams. Quilter also observed a size effect in that small crayfish only emerged after sunset whereas large adults remained active during the day. He suggested that this pattern might be explained by size-mediated predation pressure. Juvenile crayfish are likely to be prone to predation at all stages of the moult cycle, whereas large individuals are more likely to be taken only after ecdysis when the exoskeleton is still soft (Scott and Duncan, 1967).

A variation on the diel distribution pattern described above was found in a stream population of the astacid, Orconectes punctimanus in which both protection and food were gained by sheltering in water-willow beds (Rabeni 1985).

In this chapter the diet and distribution of P. zealandicus in Lake Georgina are described and discussed. The following questions were addressed:

1. What was the diet of the crayfish, and was spatial and seasonal variability in diet evident?

2. How did temperature, food quality, and crayfish size, sex and breeding condition affect diet?

3. Were seasonal and diel fluctuations in distribution evident and if so, were they affected by temperature, food quality, crayfish size, sex and breeding condition ?

2.2 Methods

(a) Sampling

Two permanent transects were set up using shore markers (stone cairns), compass bearings and plastic buoys. The transects extended about 70 metres into the lake from midway along both eastern and western shores of the lake (**Fig 1.2**). These areas were chosen in order to incorporate as wide a variety of substrates, vegetation and gradient types as possible. Along each transect, three areas corresponding to the three major substrate and vegetation types found within the lake were sampled.

Sampling was carried out over two days between the 20th and 30th of each month every two months from January 1986 to November 1986. Sampling was timed to avoid the full moon because of reported reductions in crayfish activity during such periods as a result of increased light levels (Somers and Stechey, 1986).

The sampling programme involved the collection of weed, substrate (i.e. sediment and epilithic material) and crayfish from 3 sites on each one of a pair of transects (approximately 5 metres

on either side of the permanent transect lines - Fig 1.2). Each site corresponded to one of the three community types and its position was indicated on the permanent transect by a plastic buoy. Three weed and six benthic samples were taken at each site between 0900 and 1400 hours using SCUBA.

Weed was collected by random placement of an Ekman grab (15 cm^2). Sediment was obtained from 15 cm^2 quadrats using an air pump. Epilithon was sampled by rock collection (3 large cobbles (maximum dimension 250-260 mm) collected per shallow site). Water temperature was measured at 1 m intervals on a vertical transect near the centre of the lake. Depth was measured with a depth gauge attached to SCUBA equipment. Bottom temperatures were measured also with a maximum/minimum thermometer at the time of crayfish sampling. All benthic samples were returned to the laboratory where 10 subsamples of each substrate type were taken for measurement of organic content by ashing (6 h, $450^{0}C$) after drying for two days at $70^{0}C$.

Crayfish traps were constructed from fine, plastic mesh ($1mm^2$) supported by a wire mesh ($1cm^2$) frame. They had collapsible sides and an 80 cm^2 opening (Fig 2.1). The traps were set at all sites for two 1 hour periods during each sampling visit, beginning three hours after sundown. Bait (fish remains - about 200 g of flounder) was placed within a fine mesh (mesh size $1mm^2$) envelope, sealed to prevent contamination of crayfish gut contents and wired to the centre of each trap. All individuals caught were chilled at the lake and frozen on return to the laboratory.

Fig 2.1 Trap used to sample crayfish from Lake Georgina. (sides are 80 cm in length). Note plastic buoy to left of trap and mesh container for bait in centre. Fine (red plastic) mesh is attached below coarse wire mesh.

(b) Gut contents

Gut (gastric mill) contents were examined and analysed by percentage occurrence and points systems (Elner, 1981; Wear and Haddon, 1987). Each gut was excised whole, placed in a plastic petri dish, and gut fulness was assessed by eye as empty, 1/4, 1/2, 3/4 or totally full, using a stereoscopic dissecting microscope (Olympus) with 14X magnification. Gut contents were removed to a walled, perspex slide containing a grid (with squares measuring 4 mm^2) and spread to form a thin film. Each component was noted and its contribution to gut volume was assessed as an approximate percentage (0, 10, 25, 50, 75, 100 %) and expressed as a number of points.

For example, a gut may be 50 % full with 50 % of the contents being detritus. This would be expressed as 50 % of 50, or 25 points. Thus points express the percentage of a given gut volume taken up by a particular food item. The dissection of 50 crayfish and appraisal of the gut contents during a pilot study helped standardise assessment procedures before the 2-monthly samples were taken.

To identify and assess the condition of gut contents, materials were examined with both dissecting (14 - 80 X) and compound (100 X) microscopes. The identification of plant material was facilitated initially by reference to prepared mounts of ground plant material from the lake. Gut contents of crayfish taken from each site in July, September and November were ashed (6 h, 450^{o}C) to determine site- and month-related differences in food

organic content and for comparison with data on benthic organic content.

(c) Sex, breeding condition and size.

Sex, breeding condition (females only - presence of ovarian eggs or ova on pleopods), presence of gastroliths and size (carapace length) of each animal were noted during dissections. The frequency of gastrolith occurrence at each site each month was used as an indicator of moulting periodicity. The January sample of crayfish was analysed for size classes using MLP (Maximum Likelihood Programme) on a Burroughs computer. The resulting size class categories were used subsequently when analysing the effect of size on diet and distribution.

Data on sex, breeding condition, gastroliths and size were analysed by Chi-square. Separate and interactive tests were carried out to assess the impact of these factors on observed distributions.

(d) Diel activity

Diel activity patterns were assessed in November 1986 by trapping along two transects originating at the eastern shore of the lake (Fig 1.3). The three vegetation zones were encompassed by each transect. The investigation lasted 24 hours with 6 traps (3 per transect, 2 per vegetation zone) set for one hour every

three hours (i.e. eight times in all). Each time the traps were lifted, bait was replaced. Captured crayfish were sexed, measured (carapace length), marked (patterns of small holes were punched in the telson and uropods with a 1 mm diameter leather hole punch) and released close to the point of capture. The marking procedure was instigated to investigate dispersal but no animals were recaptured during the 24 hours although 368 individuals were caught. G^2, a non - parametric approximation of Chi-square, was used to isolate and establish the significance of peaks of activity. Chi-square was used, to test the significance of sex and size ratios.

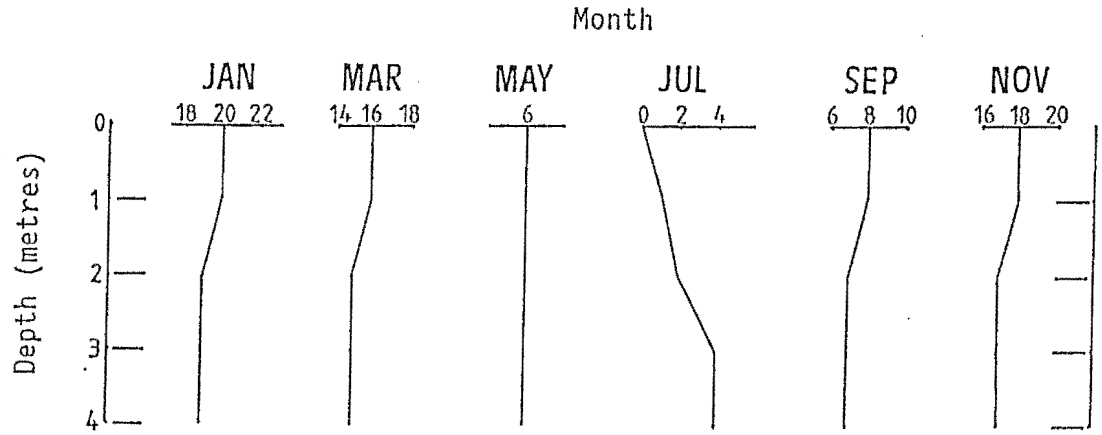
2.3 Results

2.3.1 Temperature

Water temperature varied between 0^{o} and $22^{o}C$ during the period of the study. Temperature profiles taken, using SCUBA, between 1100 and 1300 hours showed little change in water temperature with depth in any month (Fig 2.2a and b). Surface temperature was usually $1\text{-}2^{o}C$ above bottom temperatures except during May and July. In July, 4-6 cm of ice was present over the entire surface of the lake, and the temperature profile was reversed. Surface and bottom temperatures taken during crayfish sampling (about two

Fig 2.2 Monthly Water Temperatures recorded in Lake Georgina

a) Profiles: Mean temperatures (°C) taken at metre intervals to 4m below the lake surface.



b) Mean bottom temperatures taken at each site at about 2300 hours on each sampling occasion.

		Month					
		Jan	Mar	May	July	Sep	Nov
Site	Shallow	19	15	6	0	7	17
	Intermediate and Deep	19	15	6	3	7	17

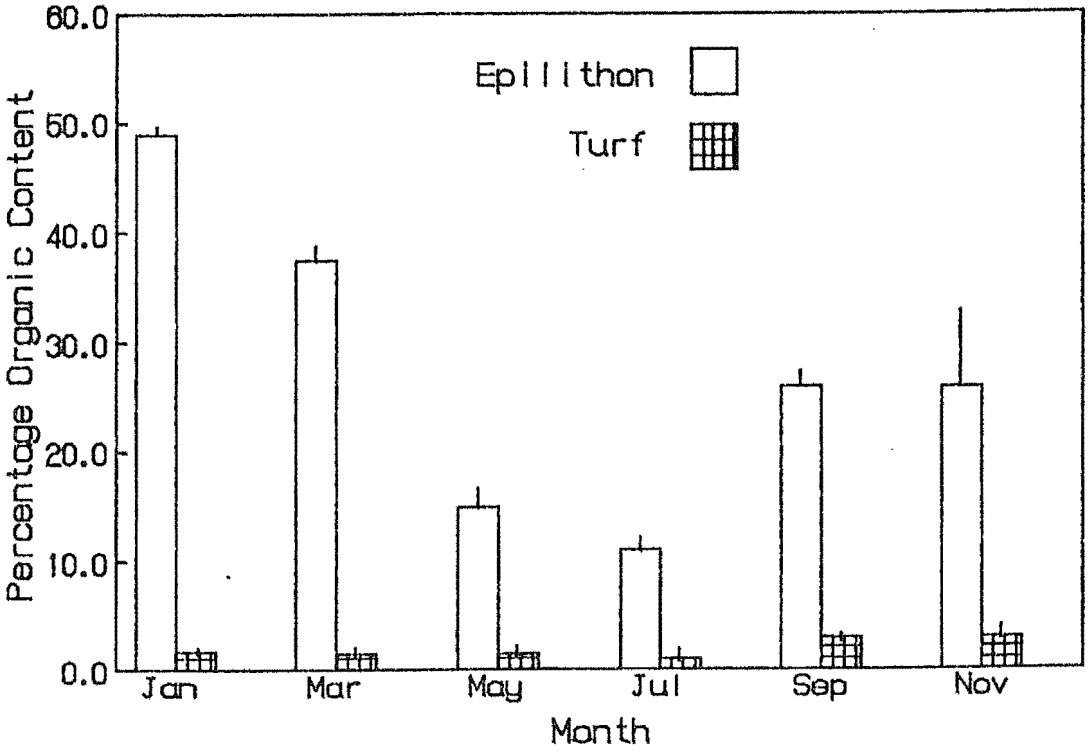
hours after sunset) were little different (usually within $\pm$ 1°C) from daytime values.

2.3.2 Organic content of benthic substrata

Monthly mean organic content (OC) of benthic sediments ranged from 8.0 to 35.0 % each month whereas monthly means for epilithon and turf were 11.4 - 51.1 % and 1.0 to 15.0 %, respectively (**Fig 2.3**). Substrate organic content was highest at the shallow sites where they were most affected by seasonal changes in temperature and photoperiod. Temperature, taken as a marker of seasonal change, was correlated significantly with shallow site total organic content (Spearmans rank (r_S) = 0.900, df=16, $P < 0.01$). Turf OC at both shallow sites generally was lower than OC at other sites during the year, and that of the east shallow (ES) site was generally lower and less variable than OC at the shallow west site. OC was highly variable at the intermediate sites (EM and WM). East deep (ED) showed generally similar, although higher, OC values to west deep (WD) and a significant decrease ($P < 0.01$, Students t) in organic content from January to May was followed by a significant peak in July ($P < 0.01$, Students t) at both of them. In September values at the deep sites were lower but they rose again in November.

Fig 2.3 Organic content (%) of substrate samples taken from Lake Georgina in 6 months. Vertical bars are 95% confidence limits.

a) East Shallow



b) West Shallow

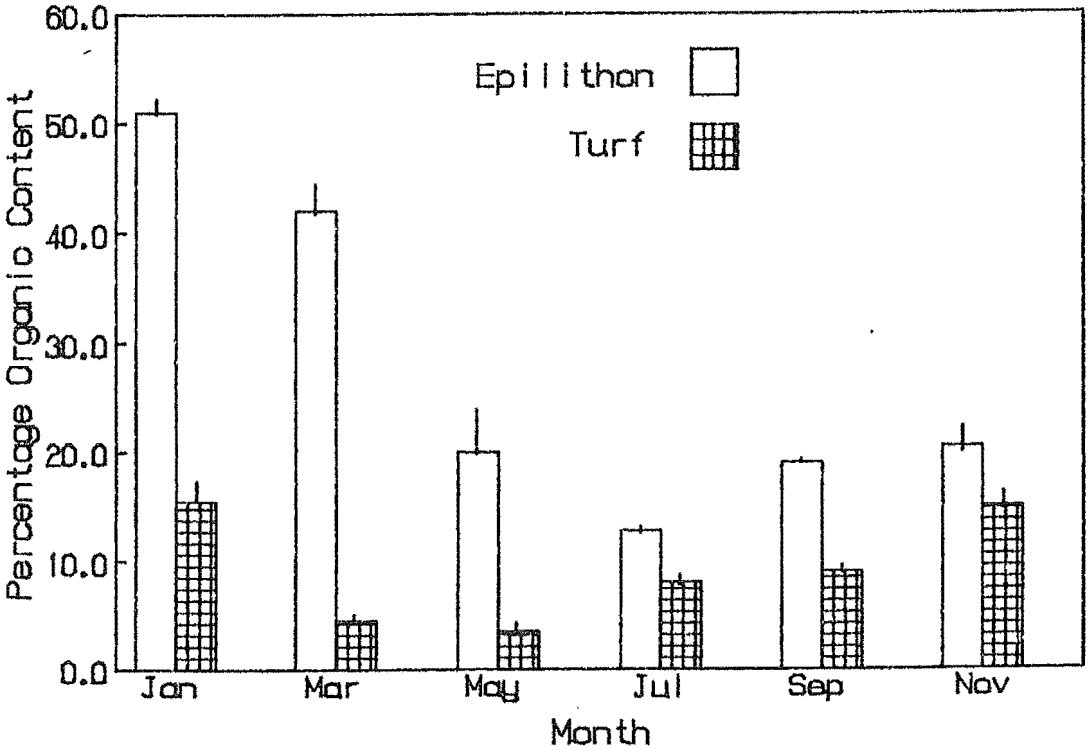
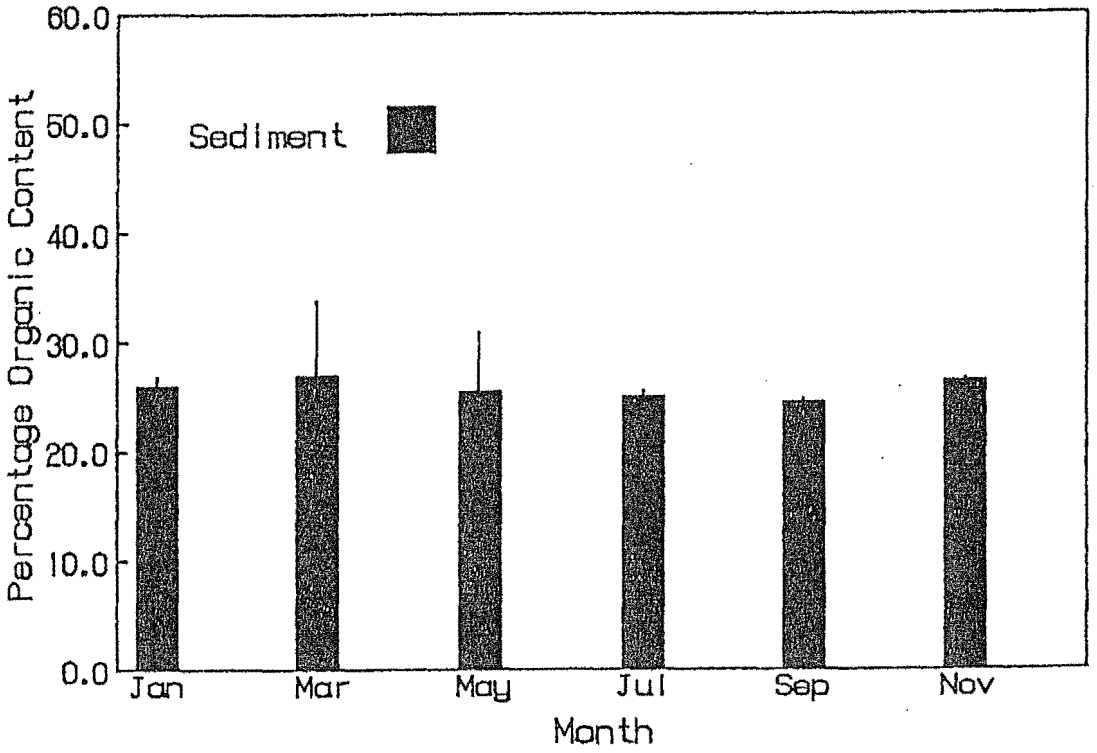


Fig 2.3 contd. Organic content (%) of substrate samples taken from Lake Georgina in 6 months. Vertical bars are 95% confidence limits.

c) East Intermediate



d) West Intermediate

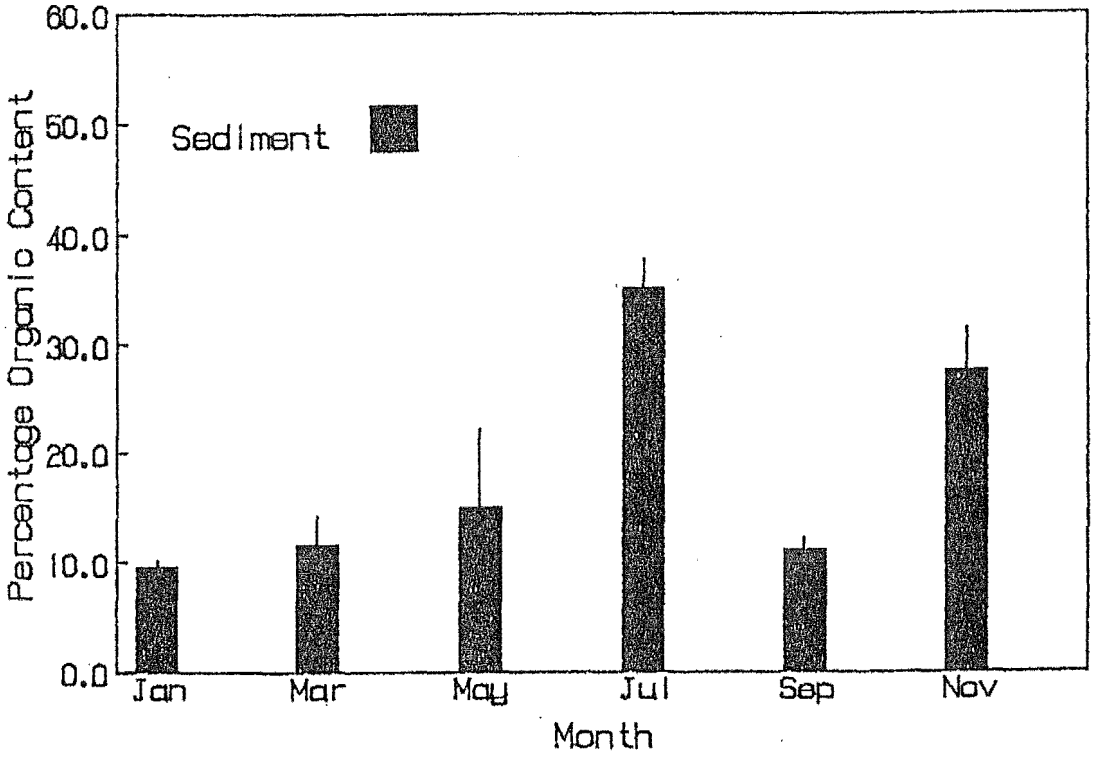
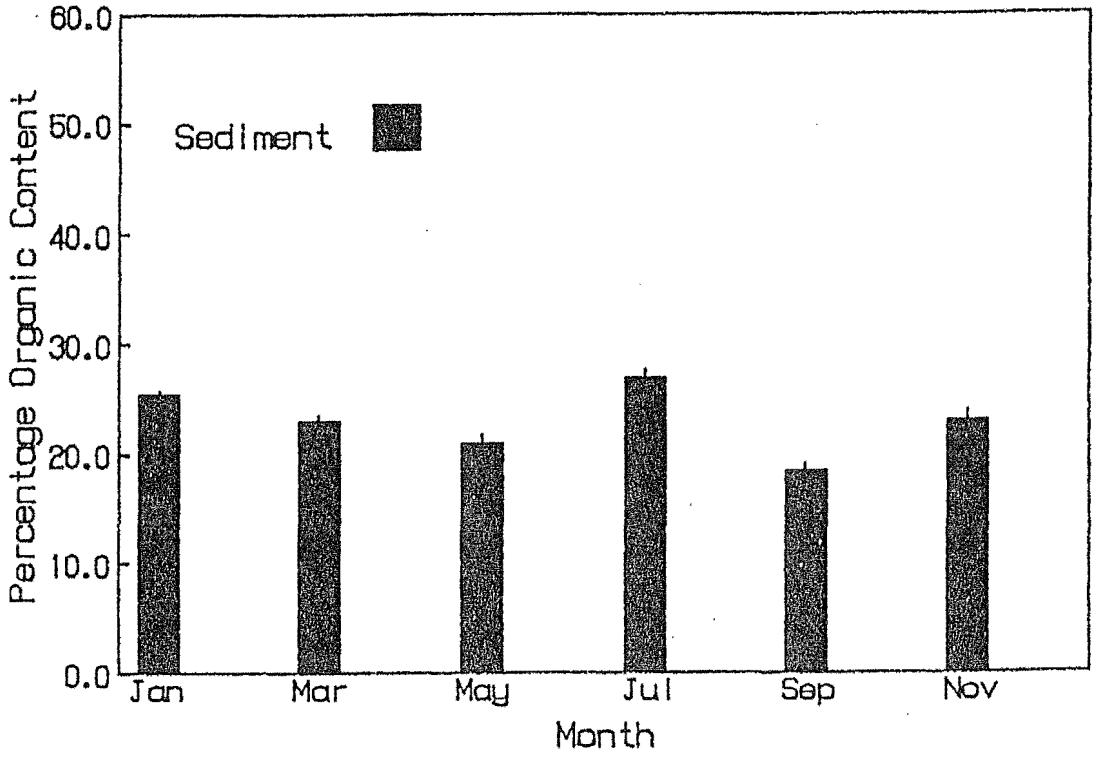
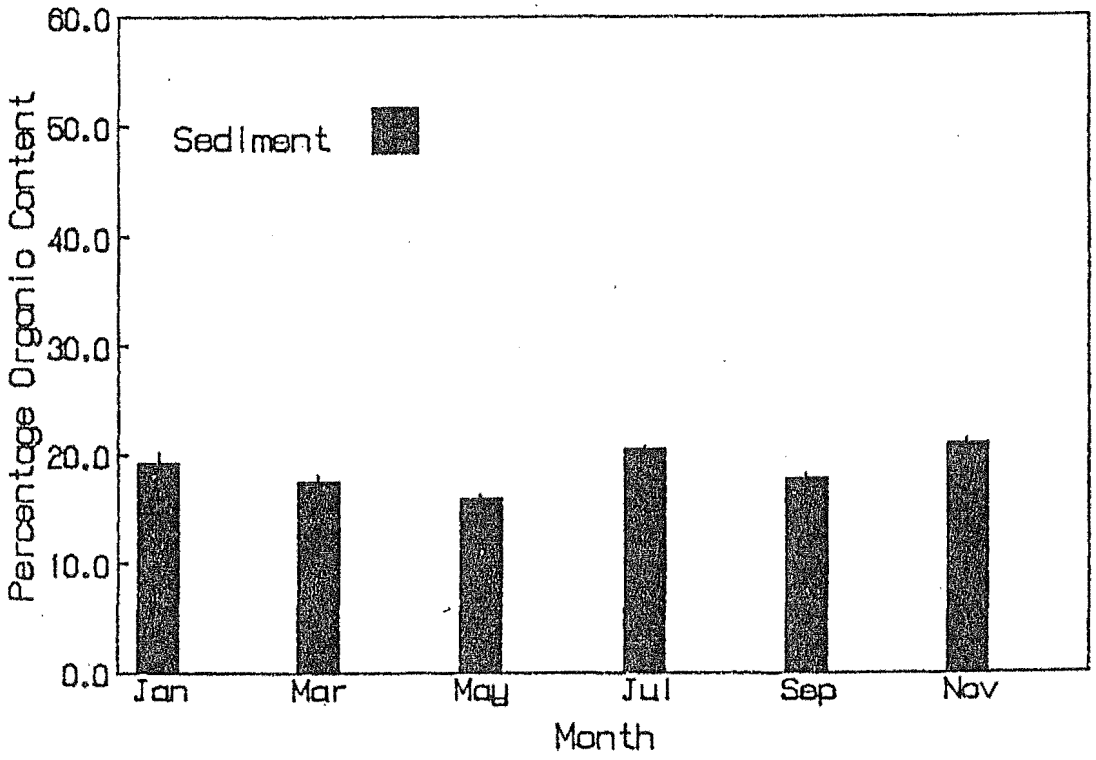


Fig 2.3 contd. Organic content (%) of substrate samples taken from Lake Georgina in 6 months. Vertical bars are 95% confidence limits.

e) East Deep



f) West Deep



2.3.3 Crayfish population structure, moulting and breeding periodicity

Six hundred and eighteen crayfish were trapped during this part of the study. Four size classes were delineated (**Table 2.1**) and used in conjunction with X^2 and G^2 to determine size/sex/distribution related effects. Size class limits were set at points of overlap of the 99 % confidence limits of each of the four significant ($P < 0.001$) groups within the size frequency distribution. The lower limits of size class 2 corresponded to the CL of the smallest mature female caught (23 mm). The largest animal caught was a 47 mm CL male (132 mm total length).

Approximately equal numbers of crayfish belonged to classes 2-4 but significantly fewer size class 1 individuals were caught (**Table 2.2**). Males and females were taken in approximately equal numbers overall (300 males:318 females, 1:1.05) although there were within size class differences in relative numbers. More size class 3 females (1:1.7) than males were netted ($X^2, P < 0.05$) whereas males made up a significantly larger proportion (1:2) of class 4 ($X^2, P < 0.05$).

The size distributions of crayfish taken in the two samples at each site on each sampling occasion were tested for homogeneity of variances (f_{max}) and equivalance of means (Students t). As no differences were found between samples ($P > 0.05$) they were pooled to improve the power of statistical tests used subsequently in the analysis of dietary and distributional data.

Gastroliths are produced prior to moulting and used as a calcium source for rapid hardening of the exoskeleton immediately

Table 2.1 Crayfish size classes as determined from the January catch using MLP. N = number of individuals in each class.

Size class	N	Mean Size (mm CL)	Range of sizes between points of overlap of 99% confidence limits
1	29	1.89	1.45 - 2.27
2	36	2.51	2.28 - 2.87
3	25	3.52	2.88 - 3.58
4	39	3.91	3.59 - 4.25

Table 2.2 Total crayfish catch: numbers of each sex and size class and statistical comparisons between numbers of males and females.

	Sex		
Size Class	Male	Female	Totals
1	40	51	91
2	78	84	162
3	73	128	201
4	109	55	164
Totals	300	318	618

Significance: $\text{Chi-sq}_{tab0.05}(df=1) = 3.84$

Comparisons	Size classes	X^2_{calc}
totals	1/2	19.93
	3/4	3.97
Male/Female	3	15.05
	4	17.23

after ecdysis (Scudamore, 1947; Scott and Duncan, 1967). Thus the presence of gastroliths in active crayfish may be used as an indication of the initiation of the moulting process. Their occurrence was noted in all months except May and July (Table 2.3) which suggests that moulting did not occur in those months. Similar results have been reported for lake and river populations of two Northern Hemisphere crayfish species living in similar climatic conditions (Momot, 1984; France 1985), and both authors found that moulting did not occur at water temperatures below 10°C.

A large proportion of the females caught in the September and November samples from Lake Georgina were carrying eggs or had developing ovaries (Table 2.4) and/or contained gastroliths. Only one breeding female contained gastroliths in September compared with 32 out of 54 (60 %) collected in November.

2.3.4 Diet and distribution

2.3.4.1 Diet

All 618 crayfish taken in the regular trapping program were dissected. The guts of 450 individuals contained ingested material and were used for subsequent dietary analysis. Gut contents consisted predominantly of organic materials (Table 2.5) and variability between sites and within sites between months was low. However, guts of crayfish from ES contained significantly more inorganic matter than those from all other sites ($P < 0.01$,

Table 2.3 Percentage occurrence of gastroliths each month

Month	N	Percentage of monthly catch containing gastroliths
Jan	129	76
Mar	92	6
May	80	0
July	44	0
Sept	139	17
Nov	134	63

Table 2.4 Numbers of breeding/non-breeding females each month and significance. Breeding was defined as the prescence of attached eggs or developing ovaries. $\text{Chi-sq}_{\text{tab}0.05}(df=1) = 3.84$.

Month	breeding	non-breeding	$\text{Chi-sq}_{\text{calc}}$	P
Jan	1	53	50.07	< 0.05
Mar	29	24	0.47	NS
May	20	14	1.06	NS
July	7	17	4.16	< 0.05
Sept	38	44	0.44	NS
Nov	53	21	13.84	< 0.05

Table 2.5 Mean gut organic content (%) (± 95% CL). Each mean was calculated from 5 subsamples taken from pooled gut contents from each site/month. n = number of animals feeding and number of guts pooled. Sites: WS - west shallow, WM - west intermediate, WD - west deep, ES - east shallow, EM - east intermediate, ED - east deep.

	Month					
	JULY		SEPT		NOV	
Site	n	Organic content (%)	n	Organic content (%)	n	Organic content (%)
WS	0	-	12	73.6 (2.5)	18	74.3 (13.0)
WM	8	86.6 (2.0)	13	85.6 (4.3)	9	82.6 (6.3)
WD	2	84.6 (1.9)	21	79.5 (1.6)	26	81.4 (7.0)
ES	3	54.5 (2.3)	6	55.0 (6.4)	4	57.0 (3.6)
EM	1	89.6 (3.4)	10	88.0 (4.3)	11	89.0 (2.4)
ED	5	85.8 (0.4)	41	79.9 (5.5)	30	81.6 (9.0)

Students t).

Twelve foods of plant and animal origin were recognised in crayfish guts (**Table 2.6**). Although most material was obviously dead, some fresh tissue was found. Plant detritus was defined as material lacking observable chlorophyll, and although material might have been broken down during digestion green macrophyte tissue observed in faeces during my assimilation experiments (Chapter 4) suggests that most tissues defined as detritus were probably ingested as such.

Identification of gut contents

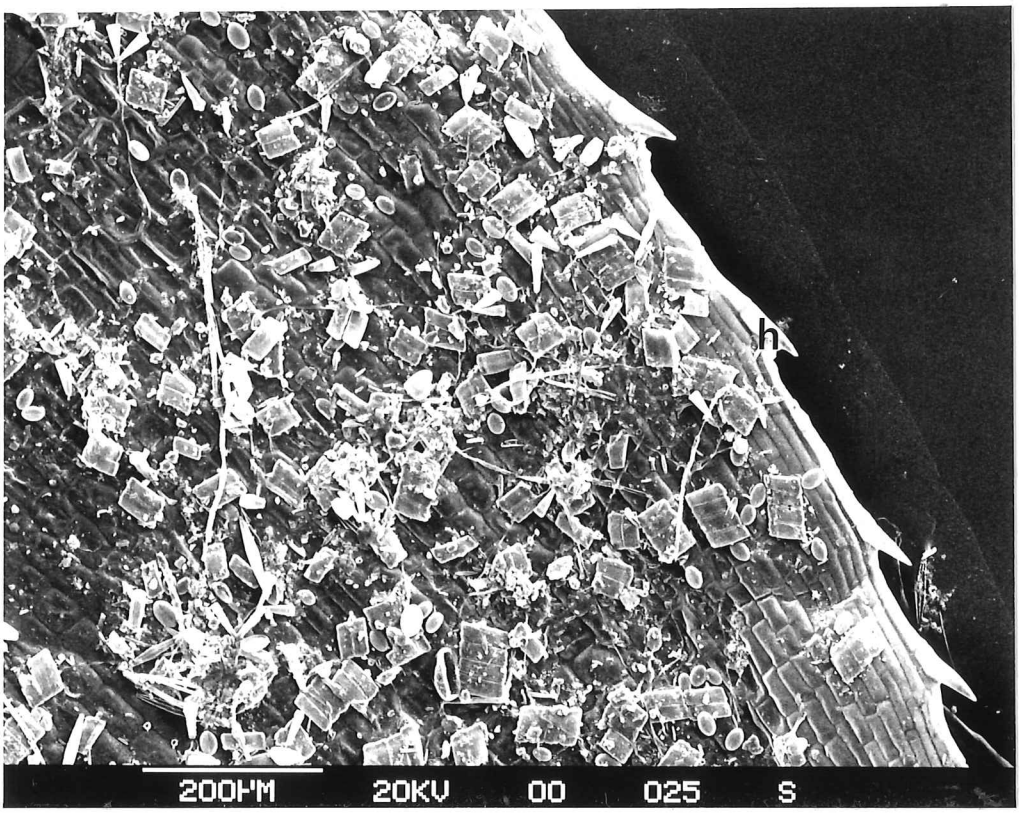
Plants were identified initially by reference to wet mounts of fresh and previously dried material. Leaves of Isoetes in guts were fibrous and flat and lacked discernible cellular detail whereas those of decayed Elodea were obviously cellular and distinguishable in turn from terrestrial leaf material by the thin leaf lamina and thin, clear, cell walls. Stems of Elodea were found occasionally and usually were associated with Elodea leaves. Leaves of this plant also bear marginal hairs which were readily identifiable in gut contents (**Fig 2.4**; Marcus et al., 1978). Chara was identified by its short pointed spikelets.

Algae were separated into epilithic and discrete filamentous categories. Epilithon was usually present in guts in clumps, and consisted of an interwoven matrix of living algal filaments, single diatoms and liverworts. Filamentous algae also occurred in discrete strands lacking chlorophyll and were categorised as detritus. The final plant category included materials that could

Table 2.6 Items occurring in the diets of crayfish in Lake Georgina from January 1986 to November 1986. Categories listed under food items are those used in subsequent analyses

Food Item	Description
a) Living Tissue	
Epilithon	filamentous and solitary diatoms, liverworts, filamentous green and blue-green algae
b) Detritus	
Filamentous algae	
Isoetes alpinus	quillwort
Chara sp.	stonewort
Elodea canadensis	aquatic macrophyte
Unidentifiable plant detritus	
Exoskeleton (crayfish)	
Crayfish (whole juvenile)	
Insects	Chironomus spp.
Other arthropods	mites (Piona uncata), ostracods (Herpetocypris pascheri)
Molluscs	Potamopyrgus antipodarum, Physa acuta, Gyraulus corinna
Fish	Upland bully (Gobiomorphus breviceps)

Fig 2.4 Electron micrograph showing marginal leaf hairs on Elodea leaves. Hairs (h) are visible on lateral margin of leaf. Scale bar = 200 um


h
200UM
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025
S

be identified as plant tissues by their cellular nature but were too fragmented to be classified further.

Initially, I planned to separate crayfish exoskeleton present in guts into two categories based on appearance; brown with and without flesh attached (evidence of cannibalism or the consumption of freshly moulted exuviae) and decayed with and without flesh attached (obtained by carrion feeding). However, no material fitting clearly into the first category was found. Fresh and decayed material was distinguished on the basis of colour which becomes more orange as decay sets in. In laboratory aquaria (17 ± 2 ^{o}C) the orange condition was noticeable after about 1 week, but it is possible that a similar colour change occurs as a result of exposure to the acids and digestive enzymes of the gut. As both cannibalism and consumption of exuviae were observed in laboratory aquaria and in the lake, and have been reported in other studies (i.e Woodland 1967 - Cherax destructor) the apparent lack of evidence for cannabalistic behaviour may have been an artifact brought about by the digestive processes. Because of these ambiguities, all crayfish exoskeletal material found in guts was grouped into a single category 'exoskeleton'.

Fragments of chironomid larvae (Chironomus spp.), mites (Piona uncata), and ostracods, (Herpetocypris pascheri) present in guts in small numbers were classified as detritus as only head capsules, parts of exuviae, and intact but empty ostracod valves were found. A single whole crayfish measuring 5.0 mm (CL) found in the gut of a larger crayfish, was the only unequivocal record of invertebrate predation. The only vertebrate remains observed in guts appeared to be those of the upland bully (Gobiomorphus

breviceps). Remains were identified from associated ctenoid scales which are found on bullies but not trout (Stokell 1955; McDowall, 1975), the only other fish recorded from the lake. Ctenoid scales differ from the cycloid scales of salmonids in having prominent spines on their exposed edges.

Analysis of gut contents

Plant material dominated the diet in all months (**Table 2.7**). It occurred in all crayfish guts and made up 87 % of the points allocated. The remaining points were contributed by animal tissue which occurred in up to 57 % of the guts in any one month. Elodea was the most important single item in the diet (**Table 2.7**). It occurred in 69 % of crayfish guts and made up 59 % of the points. Crayfish exoskeleton was the most important item of animal origin. It was found in 24 % of crayfish guts and made up 7 % of points. There was no sex or size dependent variability in diet within or between sites, transects or months.

The following spatial and temporal fluctuations in percentage occurrence and percentage points were observed for each food type:

(a) Commonly occurring foods

Epilithon was an important component of the diet at shallow and intermediate depth sites (**Table 2.8**). In the shallows it was dominant in terms of total percentage points each month and occurred in a large number (WS - 73 %, ES - 61 %) of crayfish

Table 2.7 Dietary components and their monthly contribution to food ingested. Epi - epilithon, Iso - Isoetes, Exo - exoskeleton, Char - Chara, Elo - Elodea, Ins - insects, Cray - crayfish, OA - other arthropods, Alg - algae, Mol - molluscs, UP - unidentifiable plant detritus. Y = overall % occurrence

A) % Occurrence. n = number of individuals with gut contents each month and in total.

	Food type													
	Elo	Char	Iso	Epi	Alg	Tlf	UP	Exo	Ins	Cray	OA	Mol	Fish	n
Month														
Jan	52	18	14	30	-	4	100	16	3	-	2	4	5	96
Mar	61	12	11	19	16	-	100	57	4	-	6	-	3	69
May	84	23	-	3	51	1	100	2	7	-	6	1	14	67
July	70	-	-	6	6	-	100	-	12	-	-	-	6	17
Sep	70	12	6	17	1	-	100	7	3	1	4	-	20	103
Nov	78	6	13	20	4	1	100	9	2	-	-	-	9	98
Y	69	14	8	18	12	2	100	24	4	0.2	3	1	11	450

B)% Points. N = points allocated each month and in total.

	Food type													
	Elo	Char	Iso	Epi	Alg	Tlf	UP	Exo	Ins	Cray	OA	Mol	Fish	N
Month														
Jan	48	8	4	24	-	1	6	6	0.2	-	0.1	0.7	2	8490
Mar	50	2	3	12	3	-	6	23	0.3	-	0.3	-	0.5	5965
May	69	9	-	2	3	0.1	5	5	0.4	-	0.3	0.1	6	6300
July	81	-	-	5	0.5	-	8	-	0.9	-	-	-	5	1085
Sep	62	6	1	11	0.1	-	6	2	0.2	1	0.2	-	9	9395
Nov	57	2	4	15	1	0.3	6	6	0.3	-	-	-	3	8690
Y	59	5	3	14	1	0.1	6	7	0.3	0.2	0.2	0.2	4	39925

Table 2.8 Dietary components and their contribution to food ingested at each site (all months combined). Epi - epilithon, Iso - Isoetes, Exo - exoskeleton, Char - Chara, Elo - Elodea, Ins - insects, Cray - crayfish, OA - other arthropods, Alg - algae, Mol - molluscs, UP - unidentifiable plant detritus. W - west, E - east, S - shallow, M - intermediate, D - deep, Y = overall % occurrence

A) % Occurrence. n = number of individuals with gut contents at each site and in total.

	Food type													
	Elo	Char	Iso	Epi	Alg	Tlf	UP	Exo	Ins	Cray	OA	Mol	Fish	n
Site														
WS	16	13	62	73	-	2	100	25	2	-	2	4	20	64
WM	55	38	-	12	21	6	100	8	5	2	3	-	12	65
WD	97	4	-	-	9	2	100	21	6	-	5	4	13	85
ES	10	5	-	61	-	-	100	48	10	-	-	-	19	21
EM	37	39	-	17	22	-	100	50	2	-	2	-	13	46
ED	97	3	-	-	12	-	100	20	4	-	4	-	7	169
Y	69	14	8	18	12	2	100	24	4	0.2	3	1	11	450

B) % Points. N = points allocated at each site and in total.

	Food type													
	Elo	Char	Iso	Epi	Alg	UP	Exo	Tlf	Ins	Cray	OA	Mol	Fish	N
Site														
WS	3	3	18	50	-	7	12	0.1	0.1	-	0.1	1	9	5800
WM	48	23	-	13	2	7	3	1	0.3	2	0.2	-	6	5570
WD	84	1	-	-	0.5	5	5	1	0.3	-	0.2	0.2	2	7845
ES	8	2	-	56		7	17	-	0.6	-	-	-	9	1515
EM	20	20	-	25	5	6	18	-	0.7	-	0.1	-	5	3465
ED	86	0.1	-	-	1	5	5	-	0.2	-	0.2	-	2	15730
Y	59	5	3	14	1	6	7	0.1	0.3	0.2	0.2	0.2	4	39925

guts. Consumption of epilithon (both percentage occurence and percentage points) was greatest in summer (January and November - Table 2.7) and lowest in winter (July).

Isoetes was found only in guts of crayfish caught at WS (Table 2.8) where it was a major component of the diet in terms of percentage occurrence. However, its importance in terms of points was much lower in all months (Fig 2.5). Temporal fluctuations in consumption were similar to those of epilithic material.

Crayfish exoskeleton was present in guts in all months except July (Fig 2.5). Consumption was greatest in March when exoskeleton occurred in 57 % of crayfish and it made up 23 % of the points. In other months, percentage occurrence and percentage points were generally below 10 %. Consumption of exoskeleton peaked in March at all sites except ES and WD (Fig 2.5).

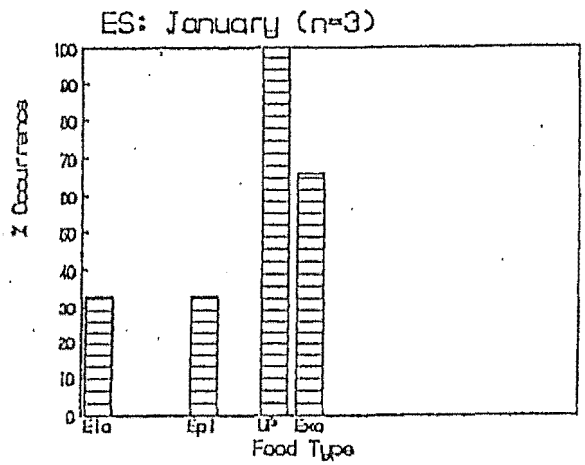
Chara and Elodea occurred in guts of crayfish taken from all sites (Table 2.8), and consumption of each was greatest in the respective weed beds. Nevertheless, the consumption of Elodea in Elodea beds was greater than the consumption of Chara in Chara beds. A general rise in Elodea consumption was observed in May, followed by a drop in percentage occurrence but a rise in percentage points in July (Table 2.7).

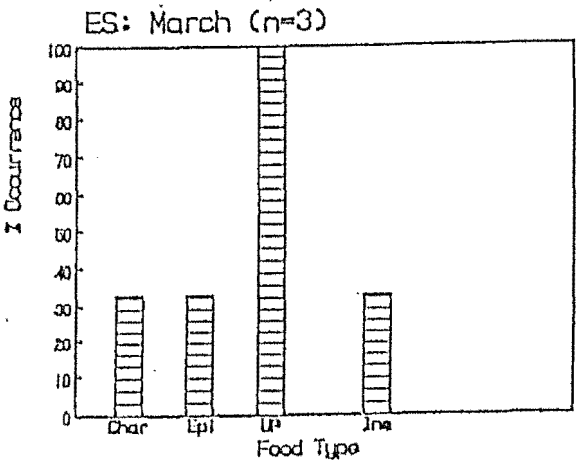
Fish tissue was found in guts of crayfish taken from all sites. Consumption reached a peak in September, especially at shallow and intermediate depth sites (Fig 2.5).

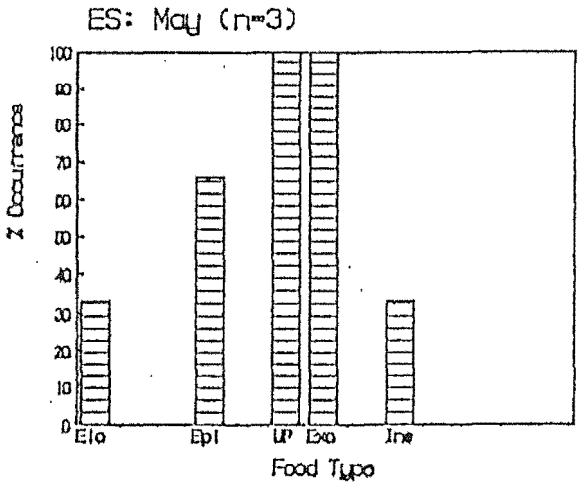
The diet of crayfish was more diverse on the western transect than on the eastern transect (Table 2.8). The guts of crayfish from WS contained the greatest number of food types and those from ES and ED contained the least.

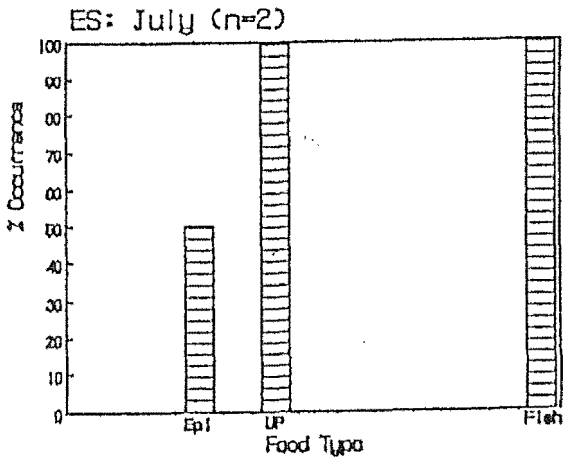
Fig 2.5 Dietary components and their contribution to food ingested (% occurrence and % points) at each site and in each month. E - East, W - West, S - Shallow, M - Mid, D - Deep. Epi - epilithon, Iso - Isoetes, Exo - exoskeleton, Char - Chara, Elo - Elodea, Ins - insects, Cray - crayfish, OA - other arthropods, Alg - algae, Mol - molluscs, UP - unidentifiable plant detritus.

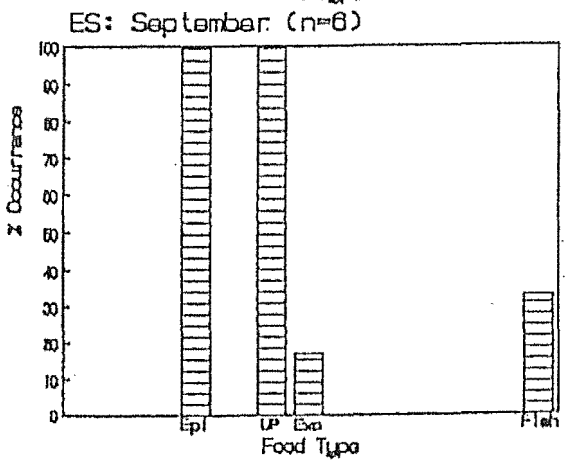
Fig 2.5.1a) East Shallow (ES): % occurrence, n = number of individuals with gut contents at each site.


ES: January (n=3)
% Occurrence
Ela
Epi
UP
Exo
Food Type


ES: March (n=3)
% Occurrence
Char
Epi
UP
Ins
Food Type


ES: May (n=3)
% Occurrence
Ela
Epi
UP
Exo
Ins
Food Type


ES: July (n=2)
% Occurrence
Epi
UP
Flesh
Food Type


ES: September (n=8)
% Occurrence
Epi
UP
Exo
Flesh
Food Type

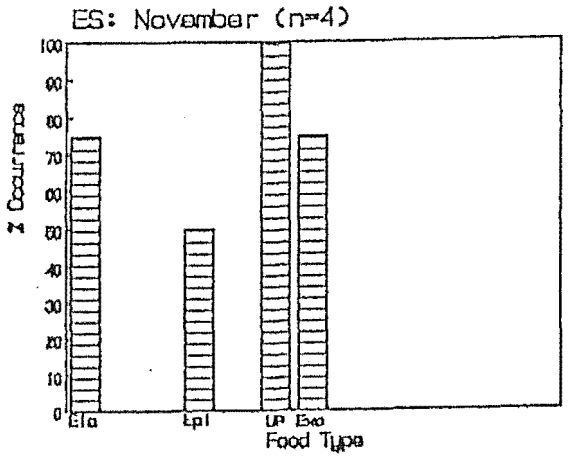
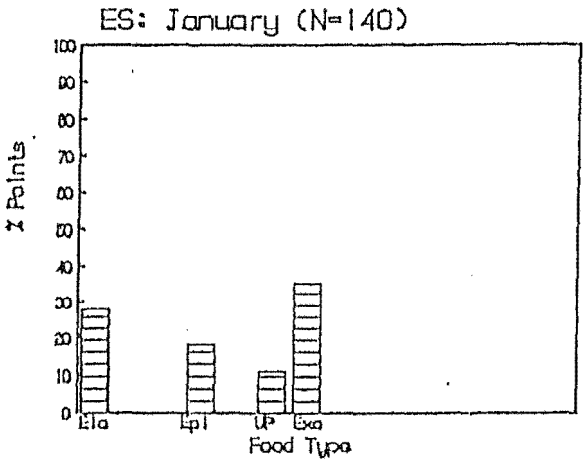
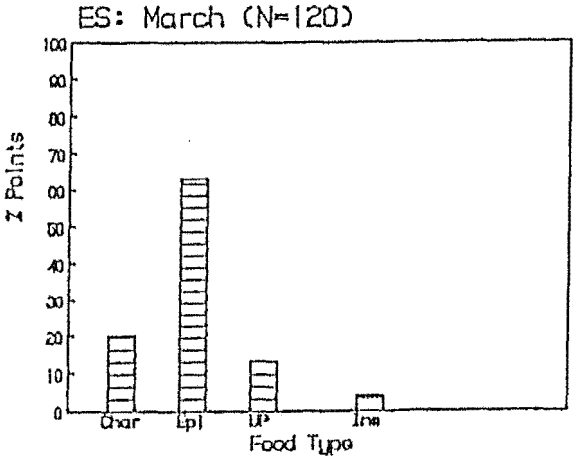
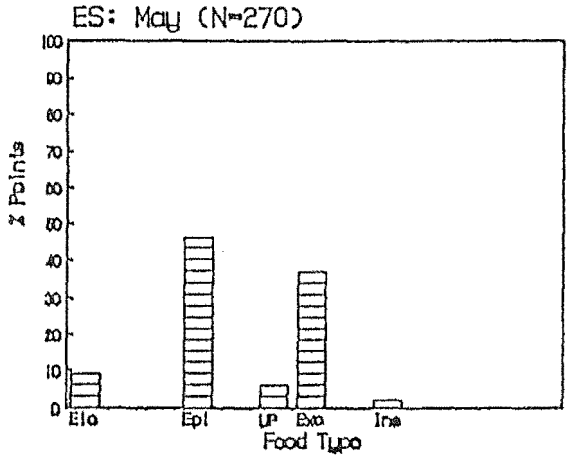
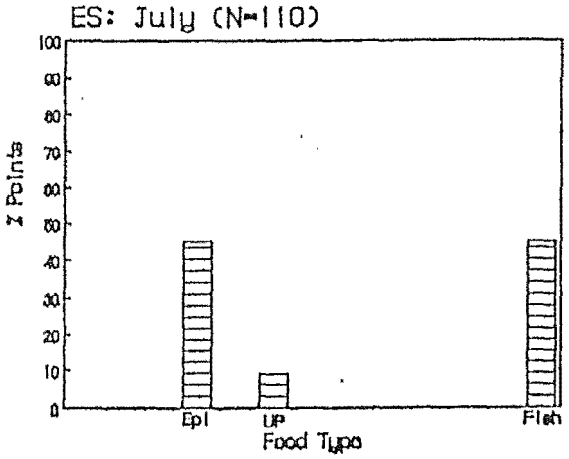

ES: November (n=4)
% Occurrence
Ela
Epi
UP
Exo
Food Type

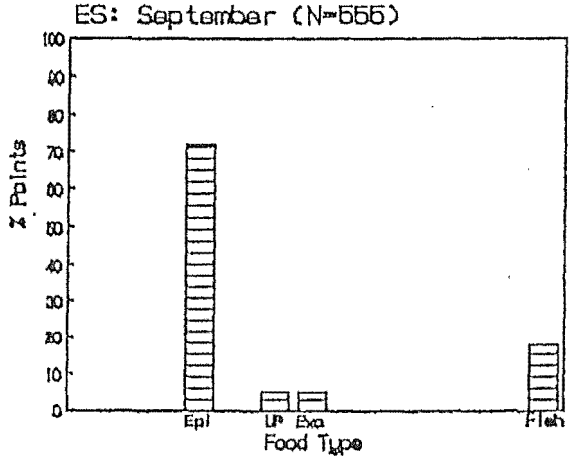
Fig 2.5.1b) East Shallow (ES): % points, N = points allocated each month. Epi - epilithon, Iso - Isoetes, Exo - exoskeleton, Char - Chara, Elo - Elodea, Ins - insects, Cray - crayfish, OA - other arthropods, Alg - algae, Mol - molluscs, UP - unidentifiable plant detritus.


ES: January (N=140)
% Points
Food Type
Ela
Epi
UP
Exo


ES: March (N=120)
% Points
Food Type
Char
Epi
UP
Ins


ES: May (N=270)
% Points
Food Type
Ela
Epi
UP
Exo
Ins


ES: July (N=110)
% Points
Food Type
Epi
UP
Fish


ES: September (N=555)
% Points
Food Type
Epi
UP
Exo
Fish

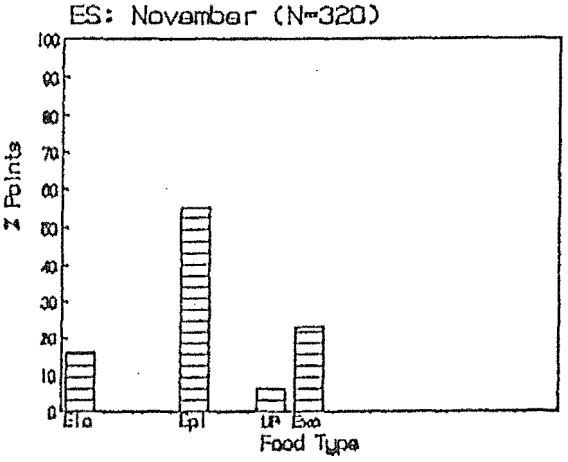
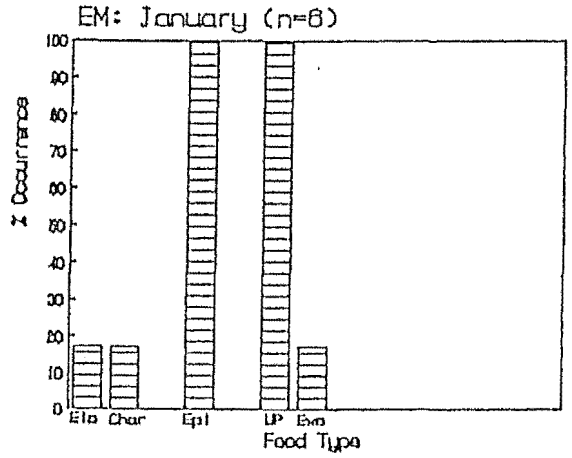
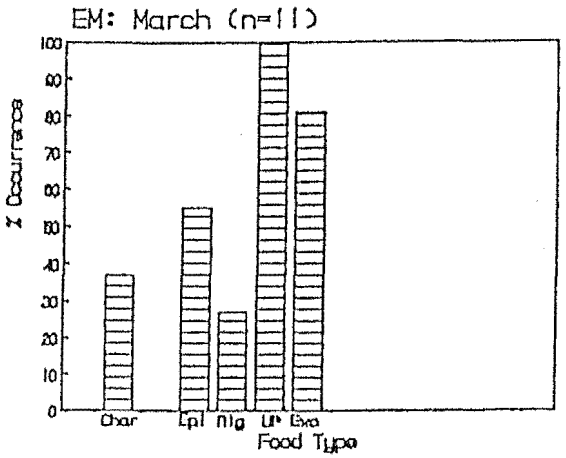
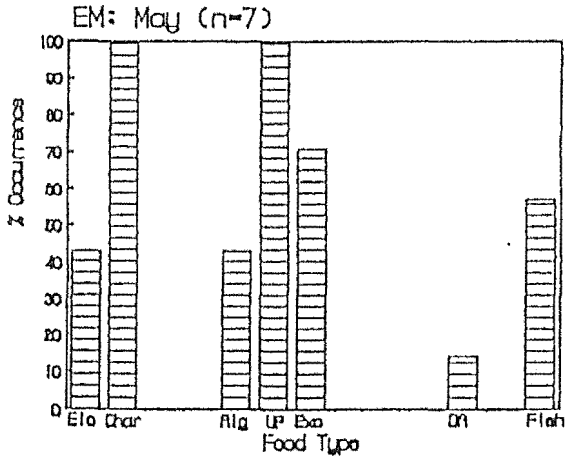
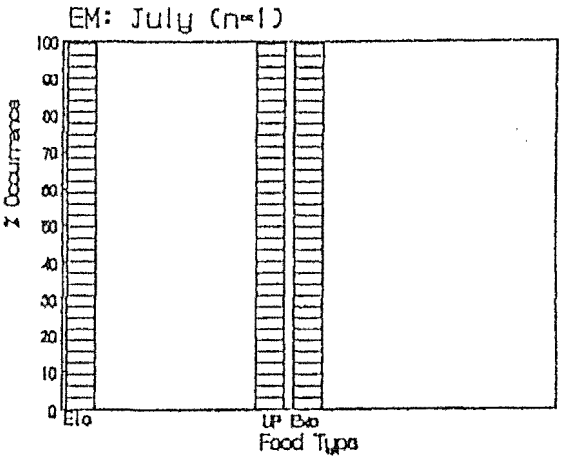

ES: November (N=320)
% Points
Food Type
Ela
Epi
UP
Exo

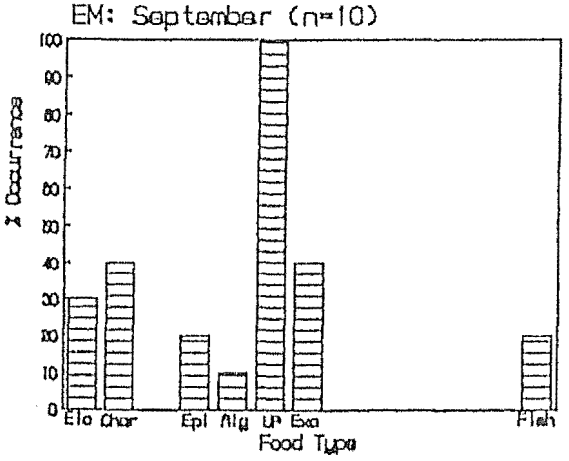
Fig 2.5.2a) East Intermediate (EM): % occurrence, n = number of individuals with gut contents at each site. Epi - epilithon, Iso - Isoetes, Exo - exoskeleton, Char - Chara, Elo - Elodea, Ins - insects, Cray - crayfish, OA - other arthropods, Alg - algae, Mol - molluscs, UP - unidentifiable plant detritus.


EM: January (n=6)
% Occurrence
Ela Char Epi UP Exo
Food Type


EM: March (n=11)
% Occurrence
Char Epi Alg UP Exo
Food Type


EM: May (n=7)
% Occurrence
Ela Char Alg UP Exo DN Fish
Food Type


EM: July (n=1)
% Occurrence
Ela UP Exo
Food Type


EM: September (n=10)
% Occurrence
Ela Char Epi Alg UP Exo Fish
Food Type

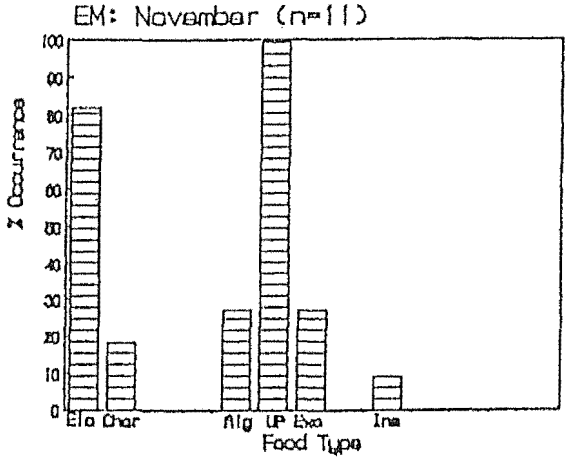
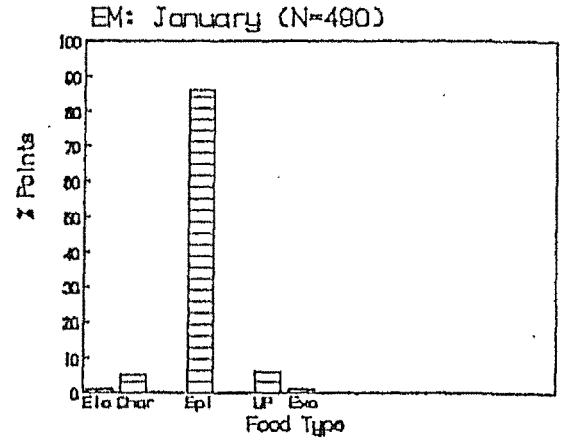
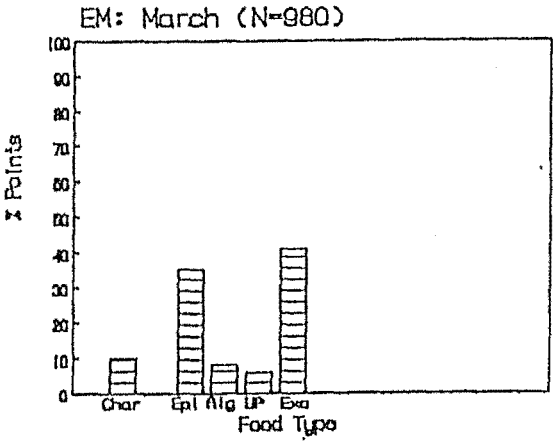
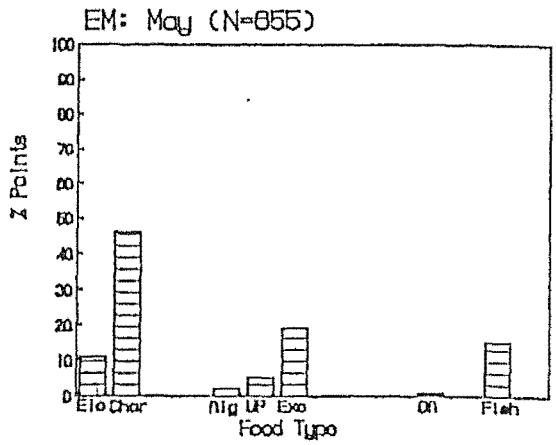
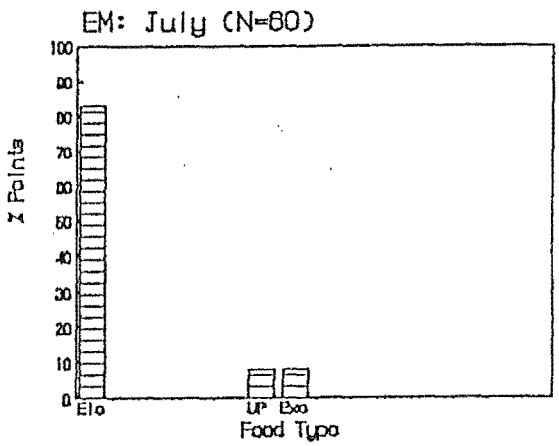

EM: November (n=11)
% Occurrence
Ela Char Alg UP Exo Ins
Food Type

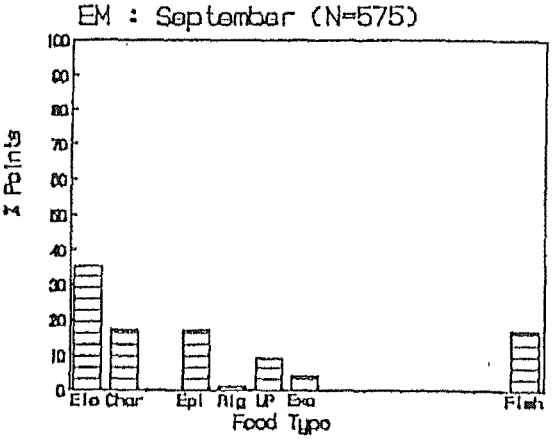
Fig 2.5.2b) East intermediate (EM): % points, N = points allocated each month. Epi - epilithon, Iso - Isoetes, Exo - exoskeleton, Char - Chara, Elo - Elodea, Ins - insects, Cray - crayfish, OA - other arthropods, Alg - algae, Mol - molluscs, UP - unidentifiable plant detritus.


EM: January (N=490)
% Points
Food Type
Elo Char Epi UP Exo


EM: March (N=980)
% Points
Food Type
Char Epi Alg UP Exo


EM: May (N=855)
% Points
Food Type
Elo Char Alg UP Exo Oli Fish


EM: July (N=80)
% Points
Food Type
Elo UP Exo


EM : September (N=575)
% Points
Food Type
Elo Char Epi Alg UP Exo Fish

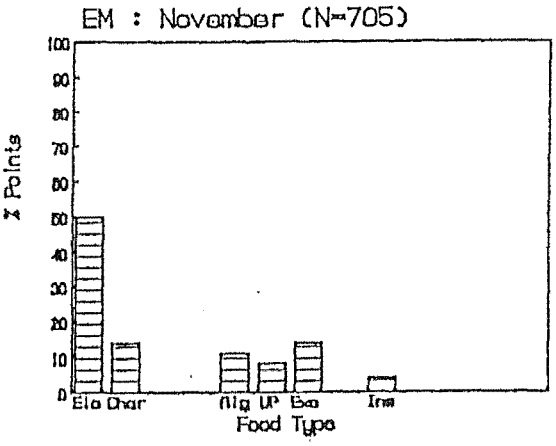
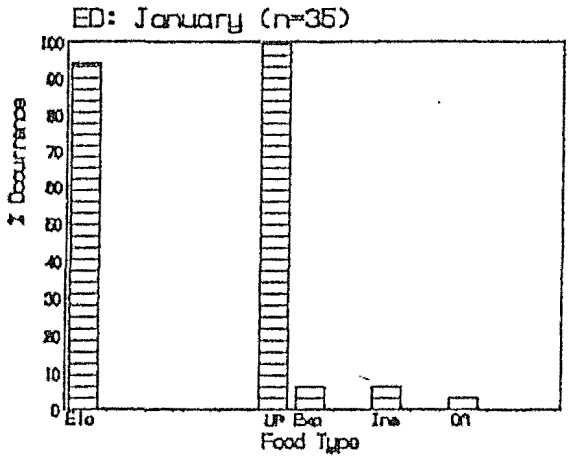
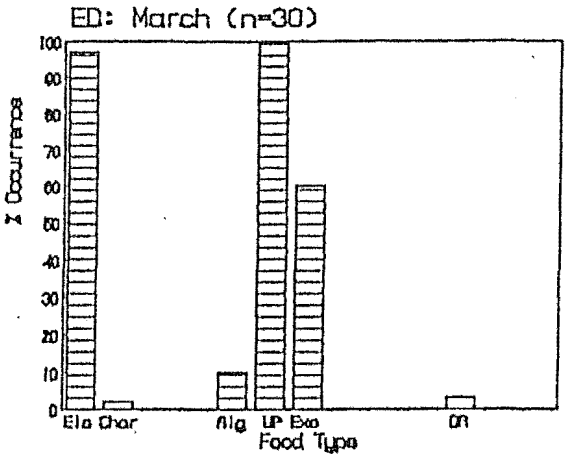
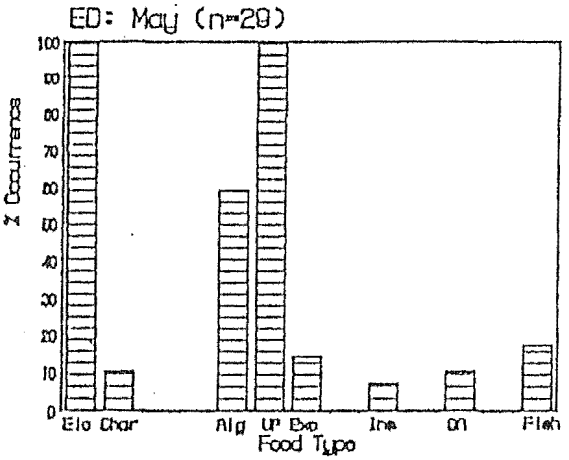
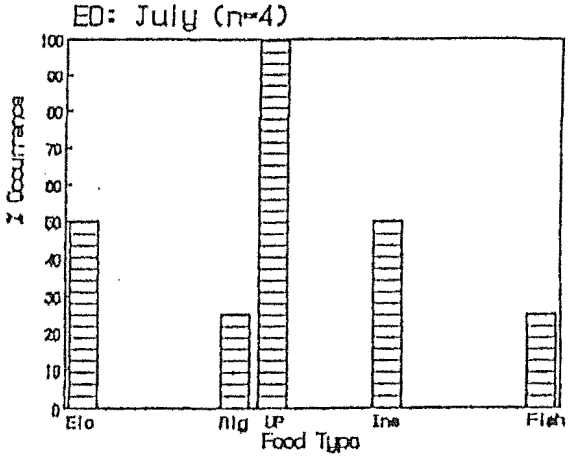

EM : November (N=705)
% Points
Food Type
Elo Char Alg UP Exo Ins

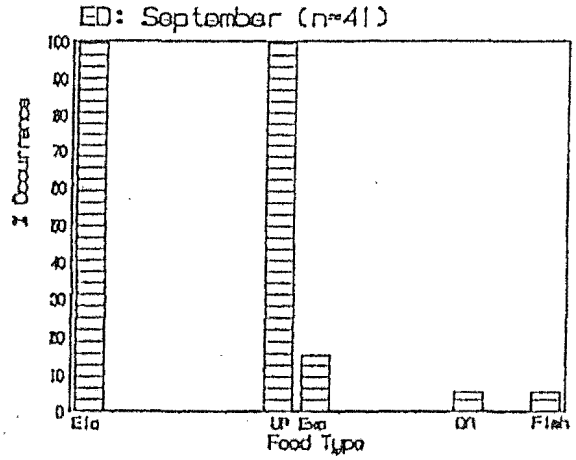
Fig 2.5.3a) East Deep (ED): % occurrence, n = number of individuals with gut contents at each site. Epi - epilithon, Iso - Isoetes, Exo - exoskeleton, Char - Chara, Elo - Elodea, Ins - insects, Cray - crayfish, OA - other arthropods, Alg - algae, Mol - molluscs, UP - unidentifiable plant detritus.


ED: January (n=35)
% Occurrence
Food Type
Elo
UP Exo
Ins
ON


ED: March (n=30)
% Occurrence
Food Type
Elo Char
Alg
UP Exo
ON


ED: May (n=29)
% Occurrence
Food Type
Elo Char
Alg
UP Exo
Ins
ON
Fish


ED: July (n=4)
% Occurrence
Food Type
Elo
Alg
UP
Ins
Fish


ED: September (n=41)
% Occurrence
Food Type
Elo
UP Exo
ON
Fish

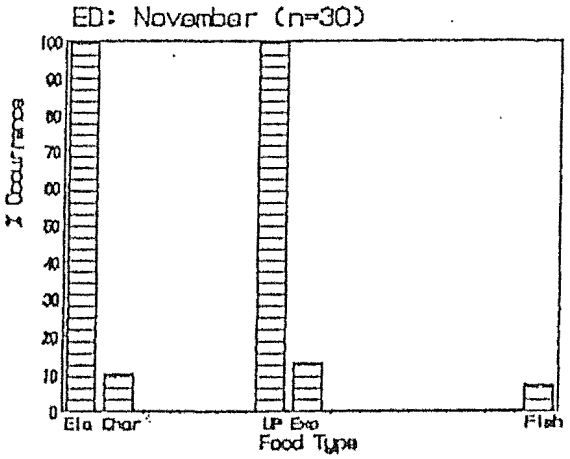
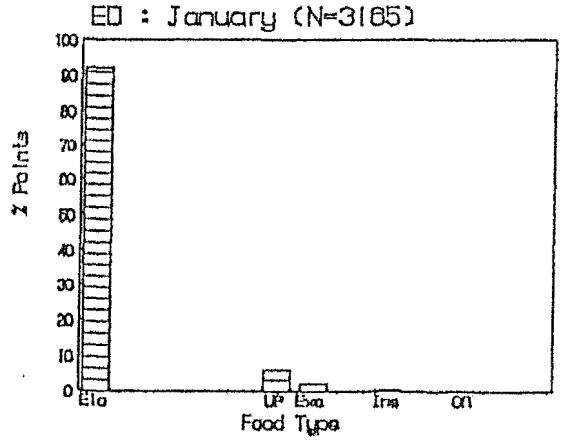
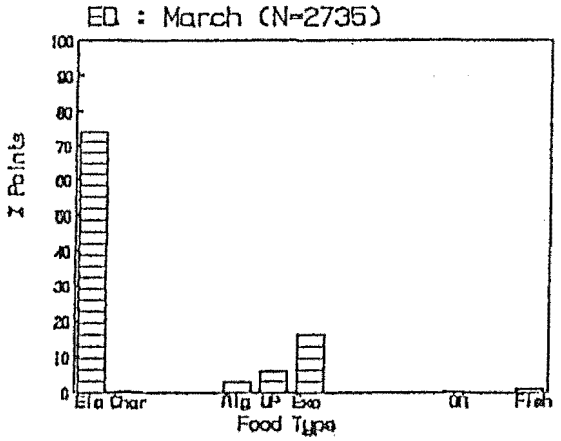
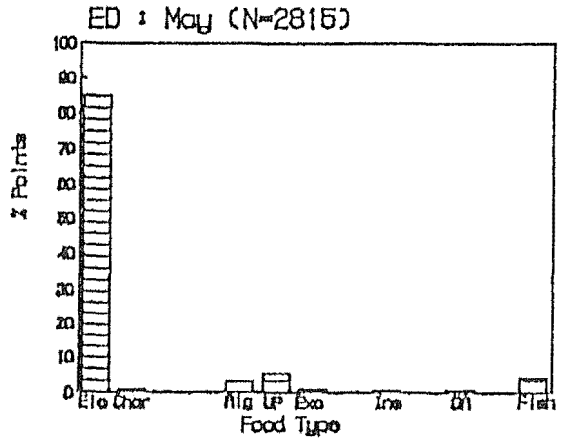
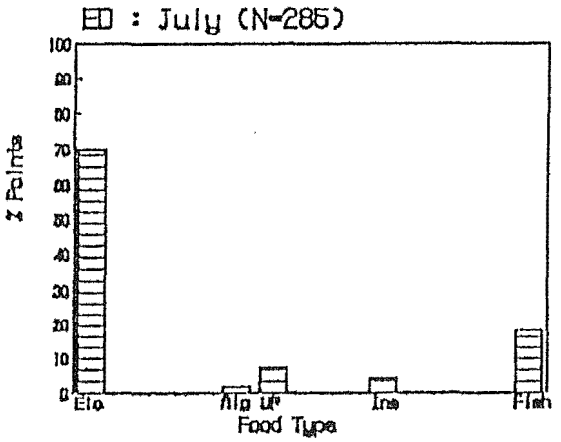

ED: November (n=30)
% Occurrence
Food Type
Elo Char
UP Exo
Fish

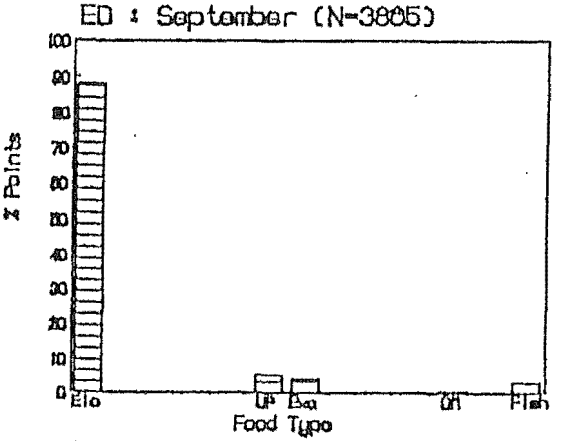
Fig 2.5.3b) East Deep (ED): % points, N = points allocated each month. Epi - epilithon, Iso - Isoetes, Exo - exoskeleton, Char - Chara, Elo - Elodea, Ins - insects, Cray - crayfish, OA - other arthropods, Alg - algae, Mol - molluscs, UP - unidentifiable plant detritus.


ED : January (N=3165)
% Points
Food Type
Ela
UP
Exo
Ins
Oli


ED : March (N=2735)
% Points
Food Type
Ela Char
Alg UP
Exo
Oli
Fish


ED : May (N=2815)
% Points
Food Type
Ela Char
Alg UP
Exo
Ins
Oli
Fish


ED : July (N=285)
% Points
Food Type
Ela
Alg UP
Ins
Fish


ED : September (N=3805)
% Points
Food Type
Ela
UP
Exo
Oli
Fish

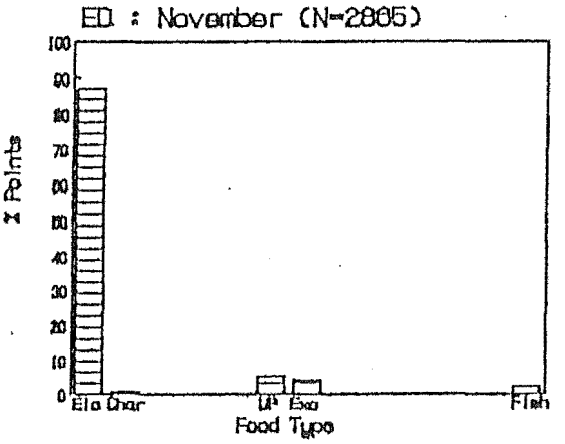
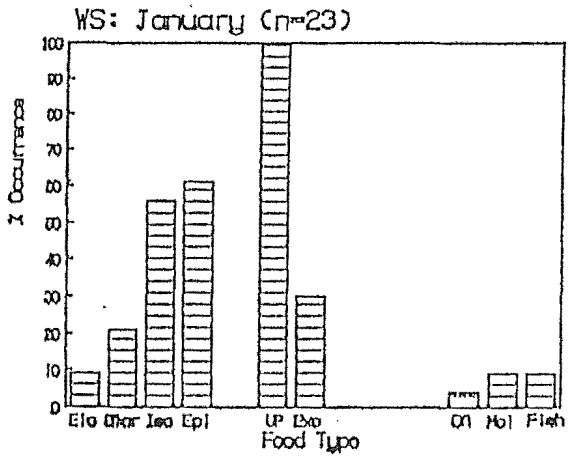
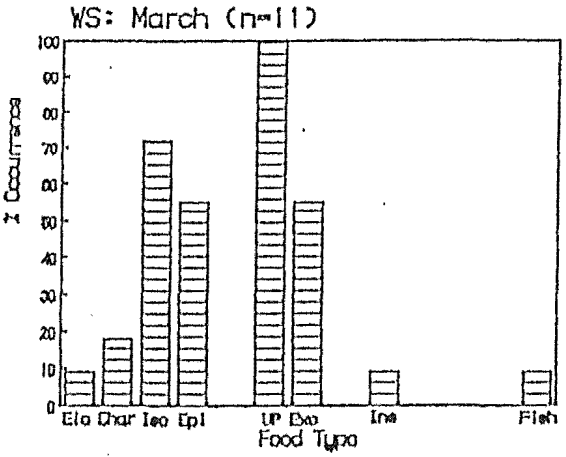
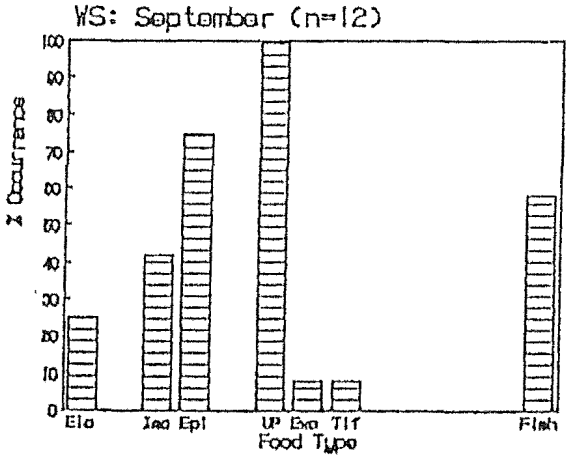

ED : November (N=2805)
% Points
Food Type
Ela Char
UP
Exo
Fish

Fig 2.5.4a) West Shallow (WS): % occurrence, n = number of individuals with gut contents at each site. Epi - epilithon, Iso - Isoetes, Exo - exoskeleton, Char - Chara, Elo - Elodea, Ins - insects, Cray - crayfish, OA - other arthropods, Alg - algae, Mol - molluscs, UP - unidentifiable plant detritus.


WS: January (n=23)
% Occurrence
Ela Char Iea Epl UP Exo Ort Hol Fish
Food Type


WS: March (n=11)
% Occurrence
Ela Char Iea Epl UP Exo Ins Fish
Food Type


WS: September (n=12)
% Occurrence
Ela Iea Epl UP Exo Tit Fish
Food Type

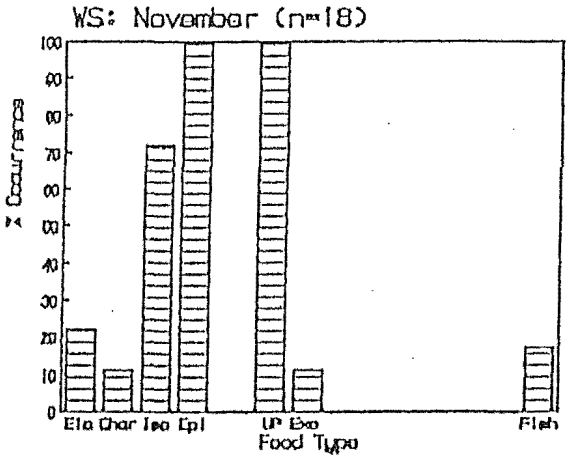
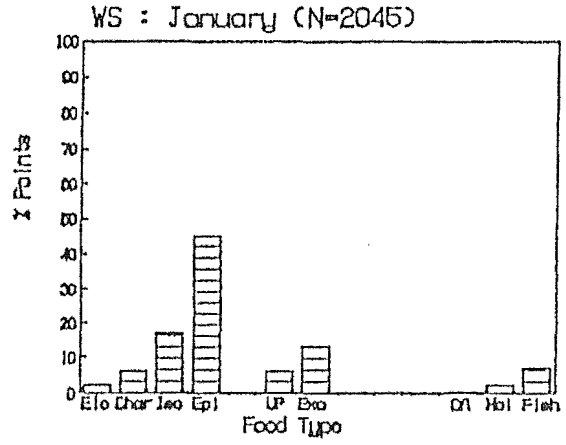
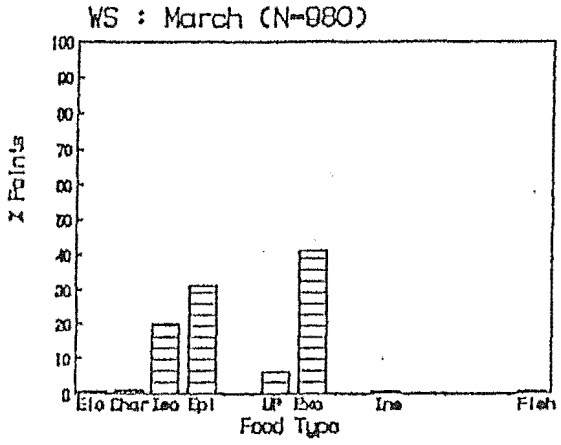
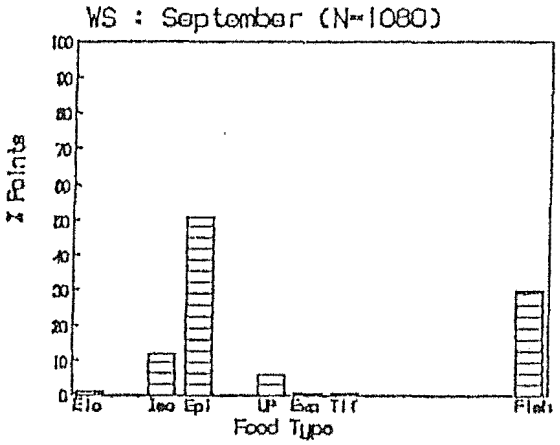

WS: November (n=18)
% Occurrence
Ela Char Iea Epl UP Exo Fish
Food Type

Fig 2.5.4b) West Shallow (WS): % points, N = points allocated each month. Epi - epilithon, Iso - Isoetes, Exo - exoskeleton, Char - Chara, Elo - Elodea, Ins - insects, Cray - crayfish, OA - other arthropods, Alg - algae, Mol - molluscs, UP - unidentifiable plant detritus.


WS : January (N=2045)
% Points
Food Type
Elo Char Ieo Epl UP Exo OA Hol Flesh


WS : March (N=980)
% Points
Food Type
Elo Char Ieo Epl UP Exo Ins Flesh


WS : September (N=1080)
% Points
Food Type
Elo Ieo Epl UP Exo Tit Flesh

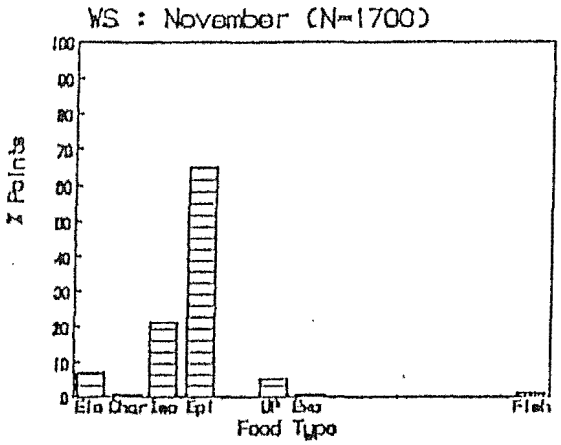
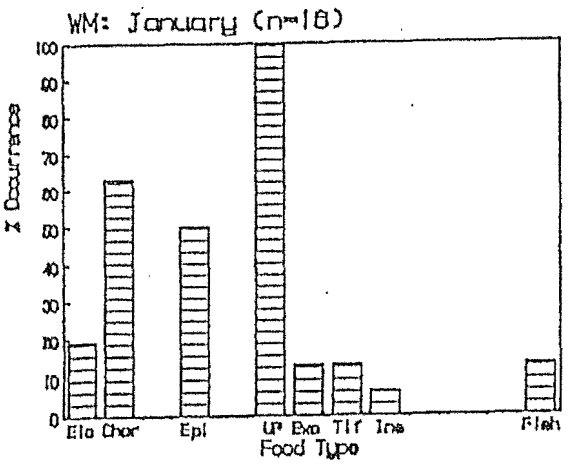
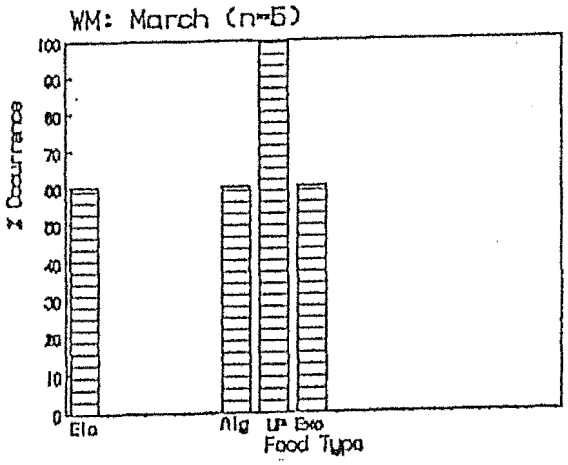
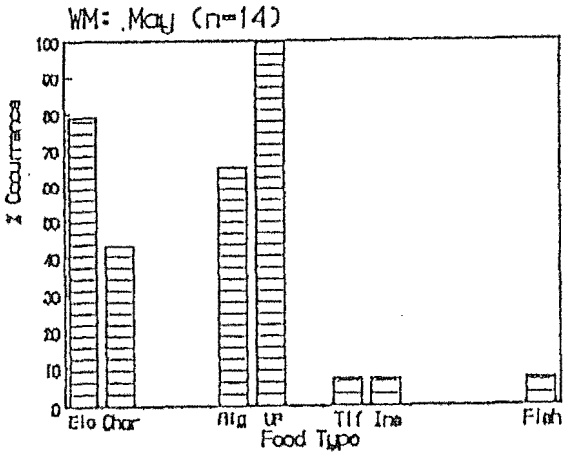
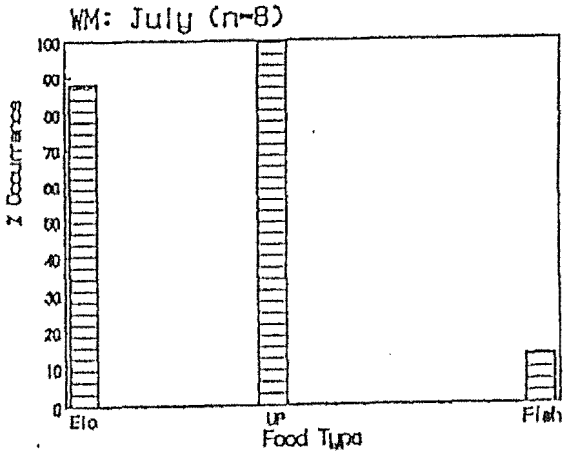

WS : November (N=1700)
% Points
Food Type
Elo Char Ieo Epl UP Exo Flesh

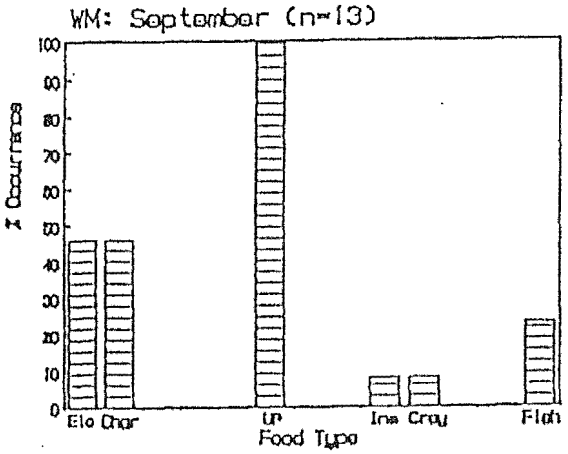
Fig 2.5.5a) West Intermediate (WM): % occurrence, n = number of individuals with gut contents at each site. Epi - epilithon, Iso - Isoetes, Exo - exoskeleton, Char - Chara, Elo - Elodea, Ins - insects, Cray - crayfish, OA - other arthropods, Alg - algae, Mol - molluscs, UP - unidentifiable plant detritus.


WM: January (n=18)
% Occurrence
Ela Char Epi Up Exo Tif Ins Fish
Food Type


WM: March (n=5)
% Occurrence
Ela Alg Up Exo
Food Type


WM: May (n=14)
% Occurrence
Ela Char Alg Up Tif Ins Fish
Food Type


WM: July (n=8)
% Occurrence
Ela Up Fish
Food Type


WM: September (n=13)
% Occurrence
Ela Char Up Ins Cray Fish
Food Type

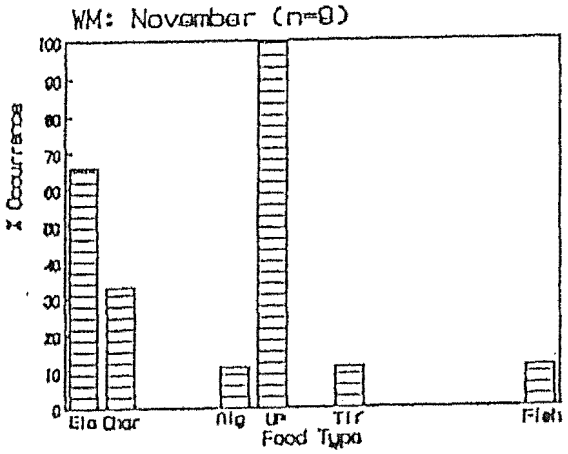
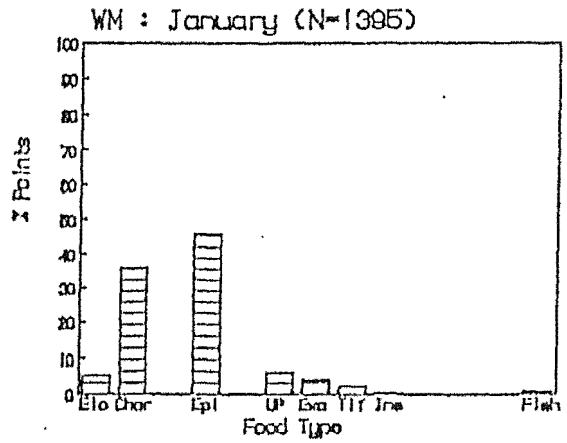
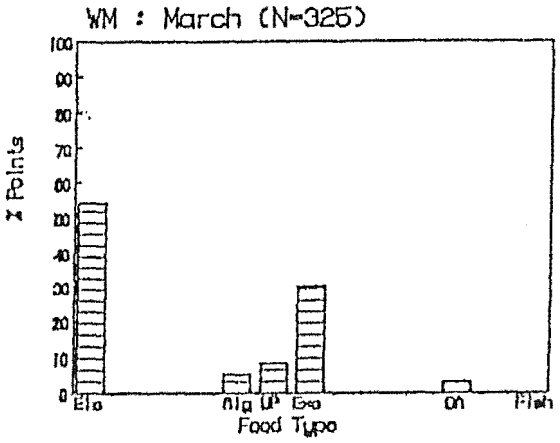
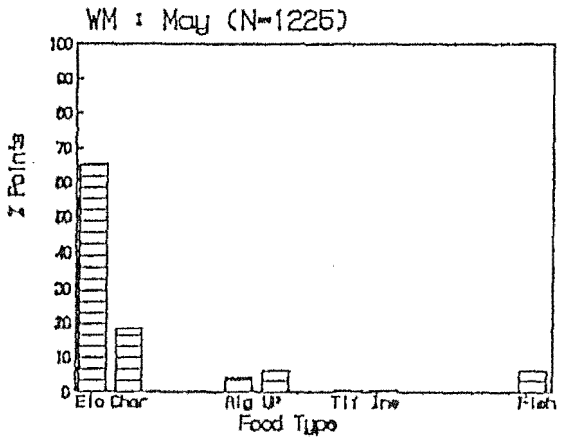
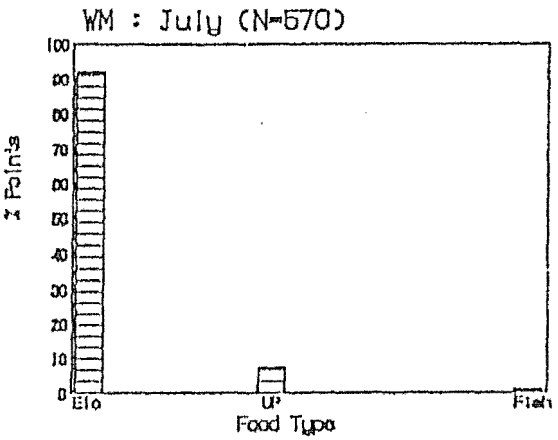

WM: November (n=9)
% Occurrence
Ela Char Alg Up Tif Fish
Food Type

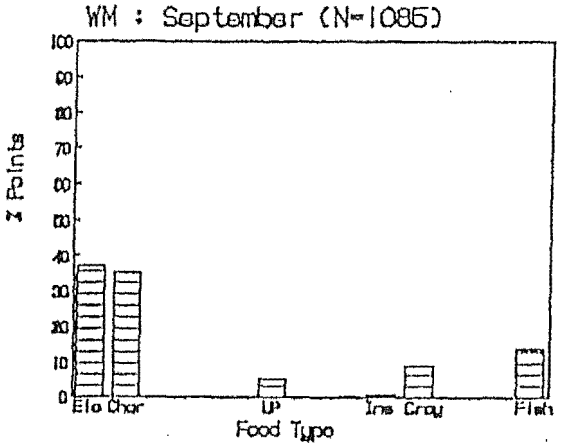
Fig 2.5.5b) West Intermediate (WM): % points, N = points allocated each month. Epi - epilithon, Iso - Isoetes, Exo - exoskeleton, Char - Chara, Elo - Elodea, Ins - insects, Cray - crayfish, OA - other arthropods, Alg - algae, Mol - molluscs, UP - unidentifiable plant detritus.


WM : January (N=1395)
% Points
Food Type
Elo Char Epi UP Eva Tif Ins Fish


WM : March (N=325)
% Points
Food Type
Elo Alg UP Eva On Fish


WM : May (N=1225)
% Points
Food Type
Elo Char Alg UP Tif Ins Fish


WM : July (N=670)
% Points
Food Type
Elo UP Fish


WM : September (N=1085)
% Points
Food Type
Elo Char UP Ins Cray Fish

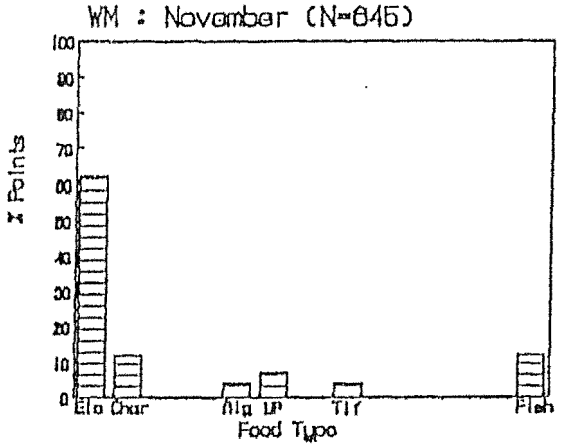
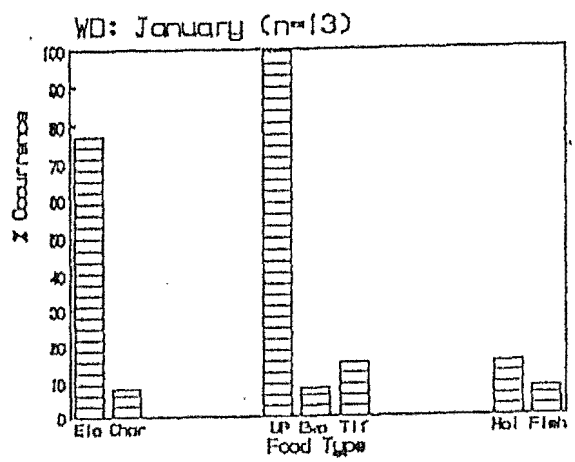
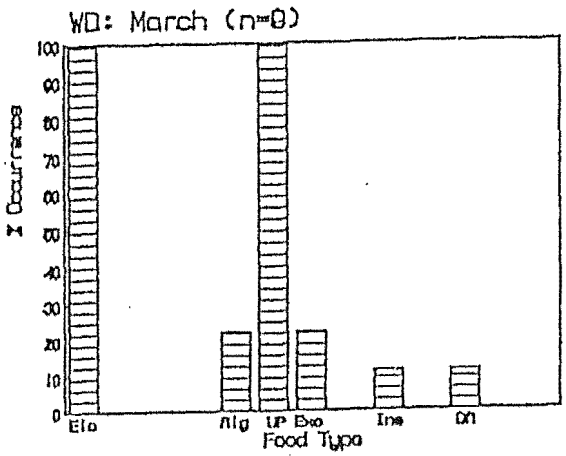
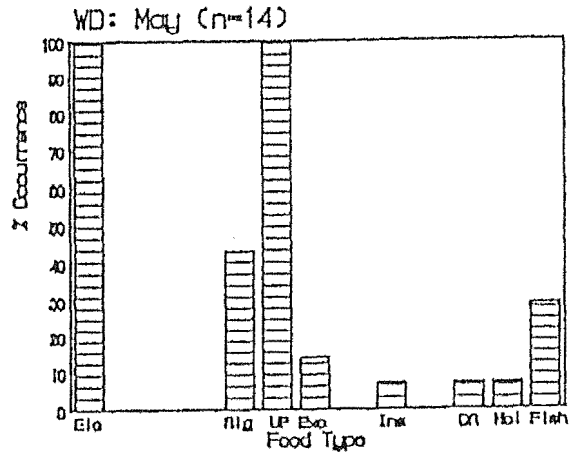
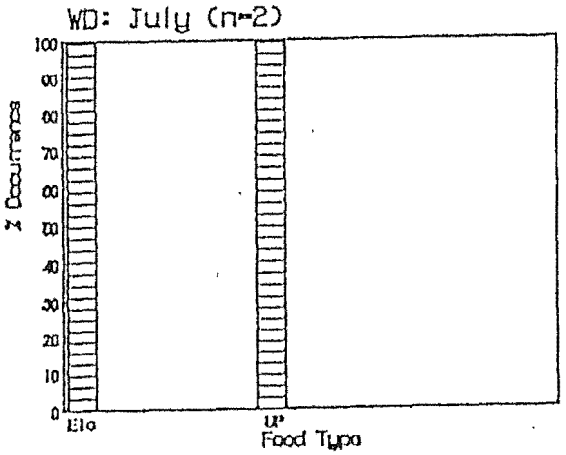

WM : November (N=845)
% Points
Food Type
Elo Char Alg UP Tif Fish

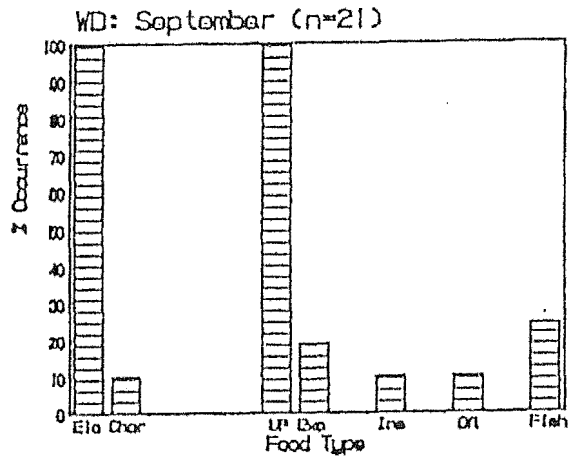
Fig 2.5.6a) West Deep (WD): % occurrence, n = number of individuals with gut contents at each site. Epi - epilithon, Iso - Isoetes, Exo - exoskeleton, Char - Chara, Elo - Elodea, Ins - insects, Cray - crayfish, OA - other arthropods, Alg - algae, Mol - molluscs, UP - unidentifiable plant detritus.


WD: January (n=13)
% Occurrence
Ela Char UP Exo Tif Hol Fish
Food Type


WD: March (n=8)
% Occurrence
Ela Alg UP Exo Ins DN
Food Type


WD: May (n=14)
% Occurrence
Ela Alg UP Exo Ins DN Hol Fish
Food Type


WD: July (n=2)
% Occurrence
Ela UP
Food Type


WD: September (n=21)
% Occurrence
Ela Char UP Exo Ins DN Fish
Food Type

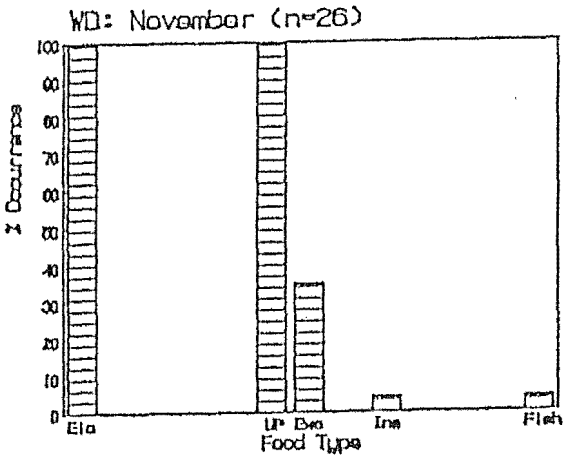
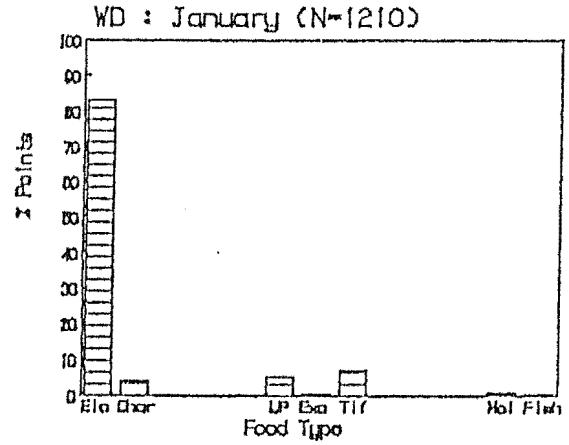
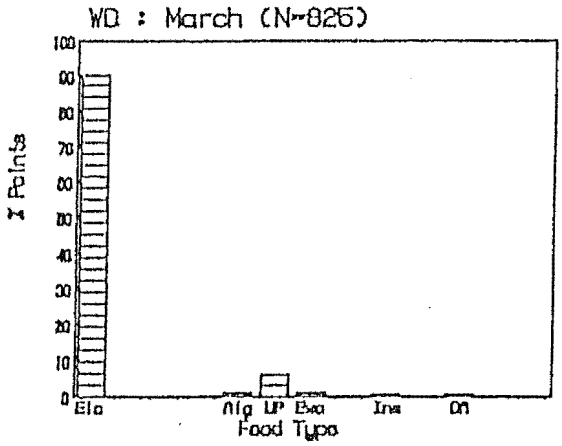
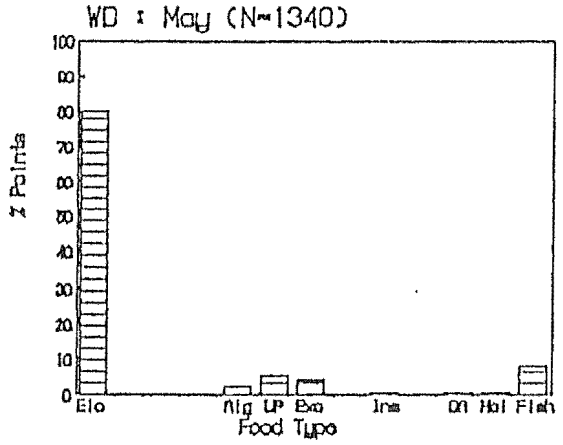
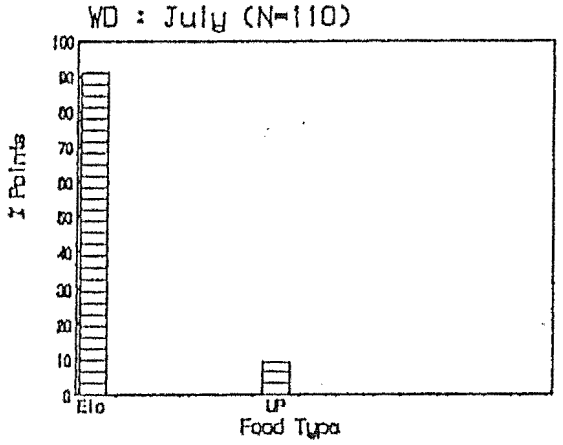

WD: November (n=26)
% Occurrence
Ela UP Exo Ins Fish
Food Type

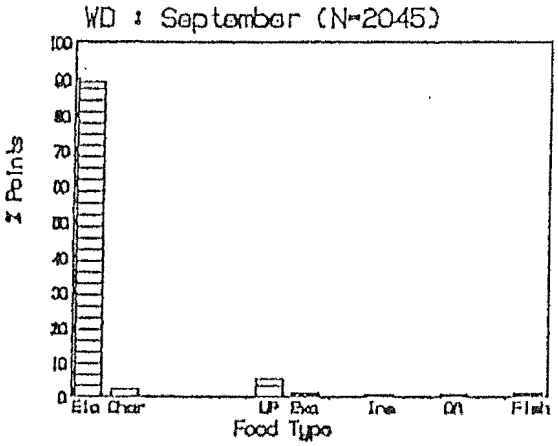
Fig 2.5.6b) West Deep (WD): % points, N = points allocated each month. Epi - epilithon, Iso - Isoetes, Exo - exoskeleton, Char - Chara, Elo - Elodea, Ins - insects, Cray - crayfish, OA - other arthropods, Alg - algae, Mol - molluscs, UP - unidentifiable plant detritus.

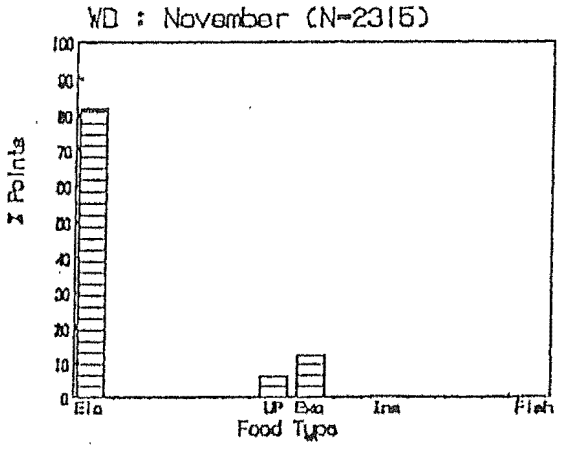

WD : January (N=1210)
% Points
Food Type
Elo Char
UP Exo Tir
Hol Fish


WD : March (N=825)
% Points
Food Type
Elo
Alg UP Exo
Ins
On


WD : May (N=1340)
% Points
Food Type
Elo
Alg UP Exo
Ins
On Hol Fish


WD : July (N=110)
% Points
Food Type
Elo
UP


WD : September (N=2045)
% Points
Food Type
Elo Char
UP Exo
Ins
On
Fish


WD : November (N=2315)
% Points
Food Type
Elo
UP Exo
Ins
Fish

b) Spatially or temporally rare foods

The consumption of insect material and filamentous algal detritus varied greatly between sites and months (**Fig 2.5**) and percentage points generally were less than half percentage occurrence values in each case. Occurrence of algae reached a peak in May at intermediate and deep sites.

The percentage points allocated to crayfish, arthropods, molluscs and terrestrial leaf material each month generally were less than 1 % of the monthly total although percentage occurrence was sometimes on a par with foods that occurred in greater volume (i.e. Chara and Elodea - **Table 2.7**). Percentage points allocated to molluscs and arthropods other than crayfish were always low (< 3 % - **Fig 2.5**) whereas those allocated to crayfish and terrestrial leaf material were generally higher.

Feeding activity

Gut fulness was used to provide a comparative index of feeding activity. Thus, allocated monthly points were compared with potential points available (including crayfish with empty guts). Total percentage points and by inference feedng activity, were highest in May (79 %) and lowest in July (25 %) (**Table 2.9**).

Table 2.9 Feeding activity each month. % points allocated = (points allocated/potential points) x 100, where potential points = number of crayfish caught x total number of possible points per individual (i.e. 100).

Month	Number of crayfish caught	Number feeding	% feeding	points allocated	% points allocated
January	129	96	74	8490	66
March	92	69	75	5965	65
May	80	67	84	6300	79
July	44	17	39	1085	25
September	139	103	74	9395	68
November	134	98	73	8690	65

2.3.4.2 Distribution

a) Temporal and spatial fluctuations in distribution and activity

Fewer crayfish were caught in July than on any other sampling occasion (**Table 2.10**). The catch decreased from May to July and increased in September and November. The largest catch was in September. Sex ratios within size classes and in different months were biased on five occasions (**Table 2.11**, $X^2_{df=1}$: $P < 0.05$). Females outnumbered males in September (all classes combined) and in March and November this was also the case for class 3 (1:3.3). More class 4 males than females (1:1.8) were caught in January and May.

Most crayfish were taken at the intermediate and deep water sites (**Table 2.12**) and abundance varied during the year, especially along the western transect. In January, crayfish numbers were higher ($X^2_{df=1}$: $P < 0.05$) at the western transect's shallow (WS) and intermediate (WM) sites than at the deep site (WD). No crayfish were taken at WS in May and July but 17 were caught there in November. At WM, numbers fluctuated widely and with no obvious temporal pattern. At the deep site on the eastern transect (ED) the catch on four of six sampling occasions was higher ($X^2_{df=1}$: $P < 0.05$) than at the other two sites (EM and ES) where numbers fluctuated widely but were generally low.

Table 2.10 Total numbers of crayfish caught on each transect and significant comparisons between months.

	Transect	
	West	East
Month		
January	71	58
March	33	59
May	37	43
July	18	26
September	64	75
November	69	65

Significant comparisons I/D - increase/decrease in catch, Chi-$sq_{tab\ 0.05}$ (df=1) =3.84,

Compared Months	Transect	I/D	X^2_{calc}	P
Jan-Mar	W	D	13.89	< 0.05
Mar-Jul	E	D	11.05	< 0.05
May-Jul	W	D	10.37	< 0.05
Jul-Sep	W	I	13.89	< 0.05
" "	E	I	21.45	< 0.05

Table 2.11 Statistically significant comparisons of sex ratios in each size class each month. M/F - comparisons between males and females. Chi-$sq_{tab0.05}$(df=1) = 3.84.

Size class	Month	M/F	X^2_{calc}
3	Mar	10/33	12.30
3	Nov	18/36	6.00
4	Jan	31/8	13.56
4	May	17/7	4.17
Total	Sept	57/82	4.50

Table 2.12 Total numbers of crayfish caught each month at each site on each transect. E - east, W - west, S - shallow, M - mid, D - deep.

	Transect					
	E			W		
	Site			Site		
	S	M	D	S	M	D
Month						
Jan	7	11	40	28	28	15
Mar	10	17	32	15	9	9
May	4	9	30	0	21	16
July	5	12	9	0	14	4
Sept	10	21	44	17	19	28
Nov	6	26	32	24	12	33

Statistically significant differences recorded in each month. $X^2_{tab0.05(df=1)} = 3.84$

Month	Comparison	No. crayfish caught at compared sites	X^2_{calc}
Jan	WS/WM	28/15	3.93
Jan	ED/EM	40/11	16.49
Mar	ED/EM	32/17	4.59
May	ED/EM	30/9	11.31
Sept	ED/EM	44/21	8.13

b) Diel activity patterns

Three hundred and sixty-eight crayfish were captured during the 24 hour experiment in November. Significantly more of the crayfish were in size classes 3 and 4 than classes 1 and 2 ($P < 0.05$, $X^2_{df=1}$, **Table 2.13**). The sex ratio was similar to that of the regular two-monthly sample taken the day before, and favoured females. The sex ratio of size classes 2 and 3 was significantly biased in favour of females ($P < 0.05$, **Table 2.13**) but there were more size class 4 males than females.

As only 20 crayfish of size class 1 were captured during the experiment, classes 1 and 2 were pooled for analysis. Temporal fluctuations in numbers caught were noted in size classes 1+2 and 3 (**Fig 2.6**). Size class 1+2 showed a significant peak in activity between 2300 and 0200 hours; most of this activity took place at the deep site. This peak preceded that at the intermediate site and it in turn preceded the increase in shallow water activity. The period of the latter was shorter than that at the other two sites. Size class 3 exhibited no differences in activity between sites but a significant activity peak ws recorded at 0200 hours ($G^2_{df=1}=41.41$, $P < 0.05$). Size class 4 showed did not exhibit any peaks in feeding activity.

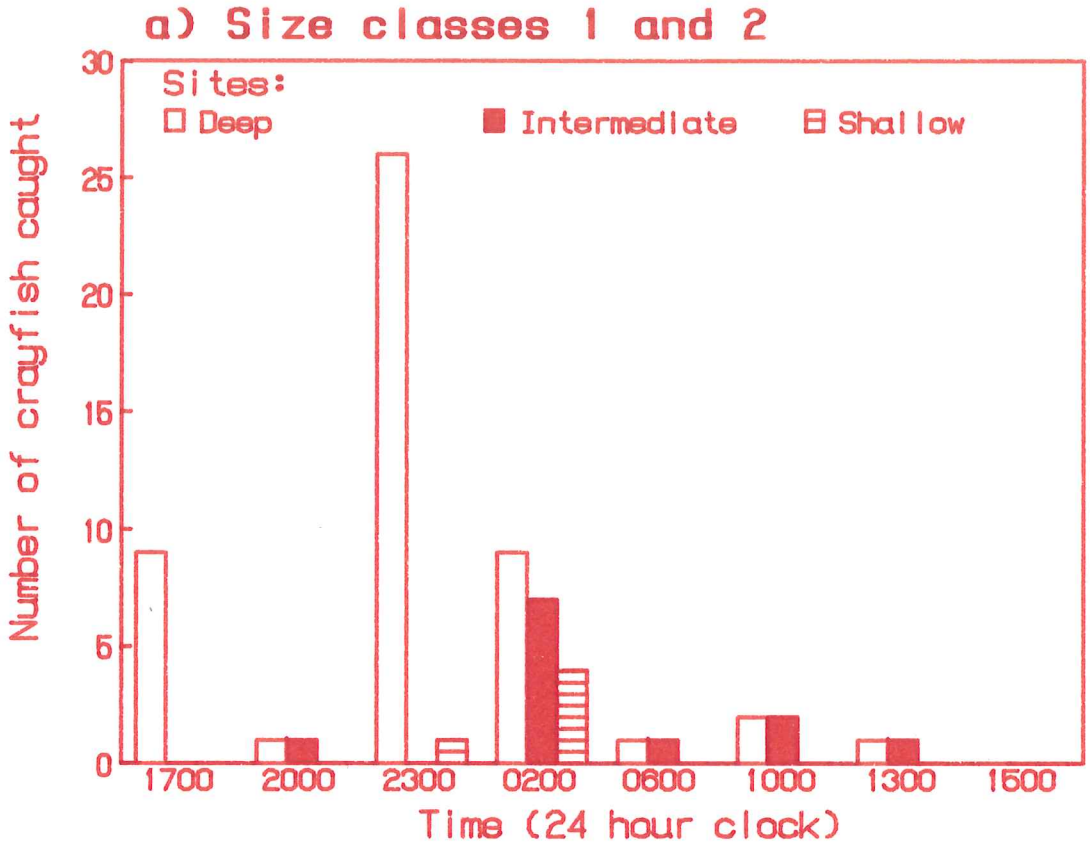
Table 2.13 Diel Activity Patterns - Total numbers of male and female crayfish in 4 size classes caught from Lake Georgina on the 28-29 november 1986.

Size class	Sex M	F	Total
1	8	12	20
2	13	32	45
3	48	110	158
4	81	64	145
Total	150	218	368

Statistically significant differences found between sexes and sizes. $\text{Chi-sq}_{\text{tab}0.05}(df=1) = 3.84$

Size Class	Comparison	No. crayfish in compared groups	X^2_{calc}
2	M/F	13/32	8.02
3	M/F	48/110	24.33
2/4	total	45/145	52.63
total	M/F	150/218	12.57

Fig 2.6 Diel activity patterns of crayfish on 28-29 November 1986. Class divisions are detailed in Table 2.1.


a) Size classes 1 and 2
Sites:
Deep
Intermediate
Shallow
Number of crayfish caught
0
5
10
15
20
25
30
1700
2000
2300
0200
0600
1000
1300
1500
Time (24 hour clock)

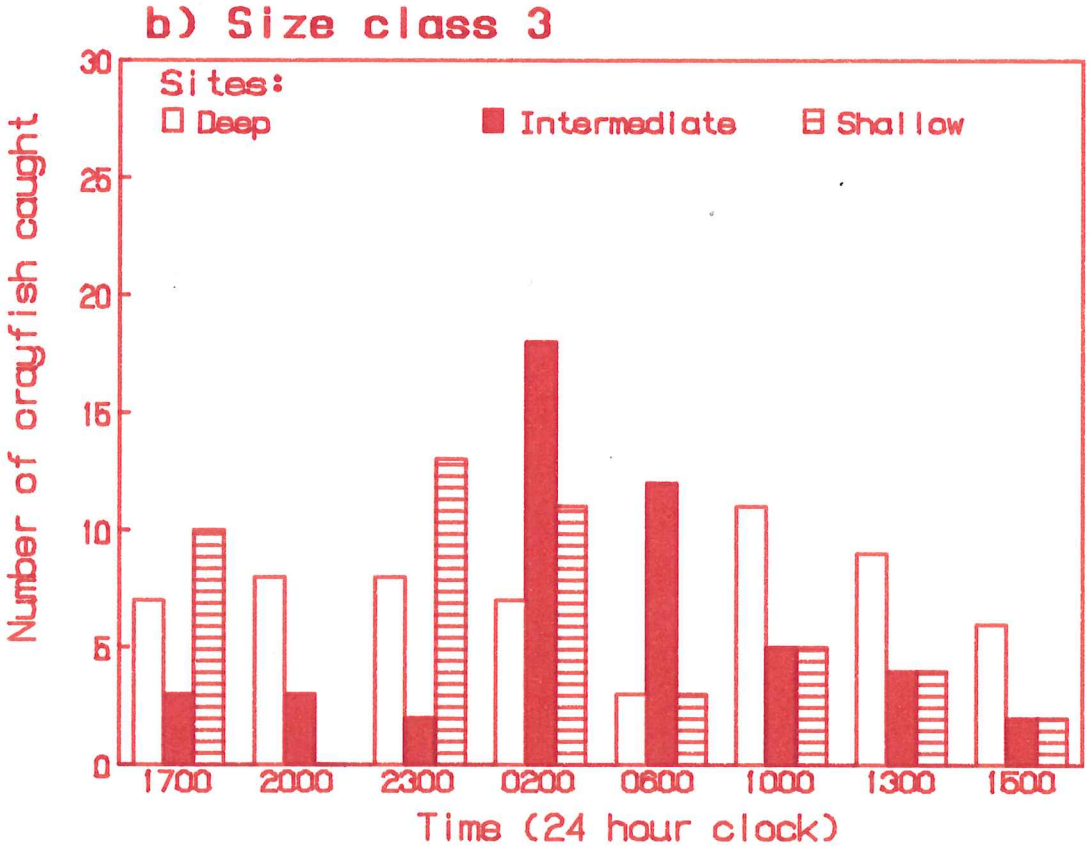
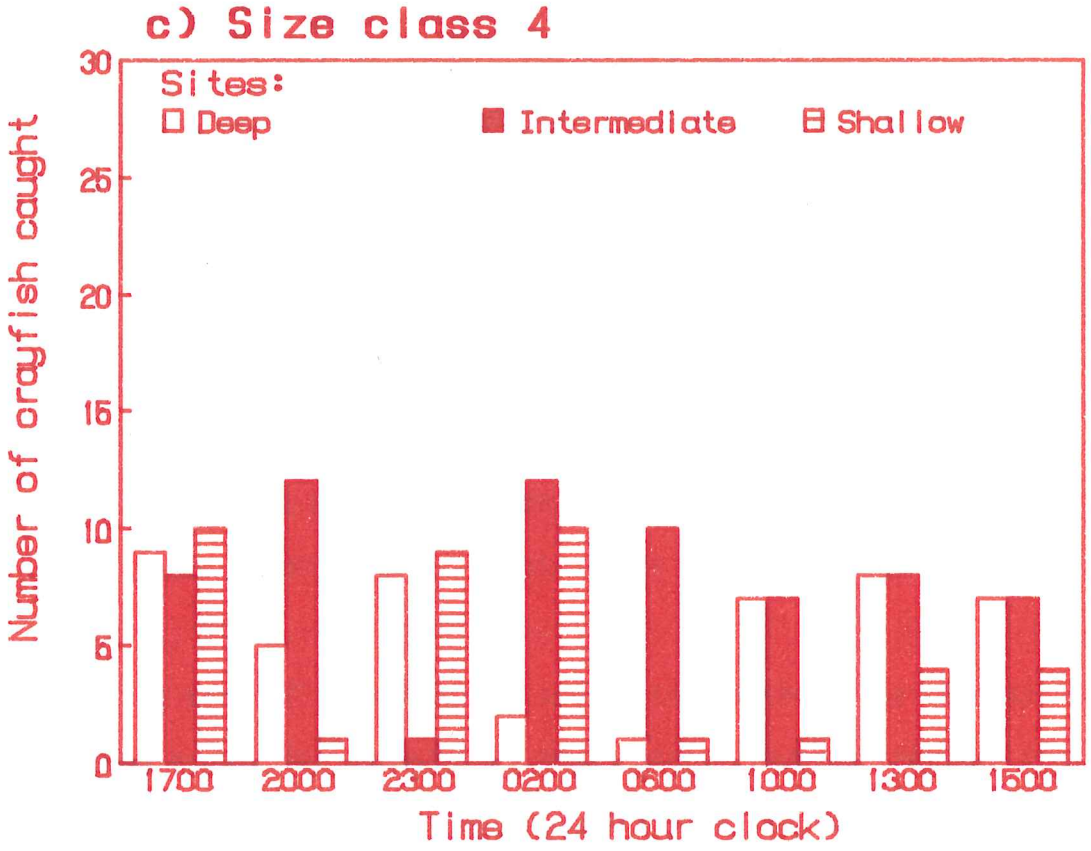

b) Size class 3
Sites:
Deep
Intermediate
Shallow
Number of crayfish caught
0
5
10
15
20
25
30
1700
2000
2300
0200
0600
1000
1300
1500
Time (24 hour clock)

Fig 2.6 contd. Diel activity patterns of crayfish on 28-29 November 1986. Class divisions are detailed in Table 2.1.


c) Size class 4
Sites:
Deep
Intermediate
Shallow
Number of crayfish caught
0
5
10
15
20
25
30
1700
2000
2300
0200
0600
1000
1300
1500
Time (24 hour clock)

2.5 Discussion

2.5.1 Method

Sampling the crayfish in Lake Georgina was complicated by the existence of two distinctly different substrate types and three different vegetation types. Ideally, SCUBA surveys of distribution would have been used in conjunction with trapping (Somers and Stechey, 1986) but the density of the Chara beds made observation of crayfish impossible in that zone, and therefore trapping alone was used in all areas to provide comparative data.

In the following discussion, catch frequencies are used to infer activity and distribution patterns, during the hours of darkness. This method has been used in a number of Northern Hemisphere studies (e.g. Momot, 1967; Momot and Gowing, 1972; Capelli, 1985).

The kind of trap used may affect the catchability of crayfish. Minnow traps, for example may bias catches towards crayfish of particular sizes, sex or breeding state (Malley and Reynolds, 1979).

Platform traps were used in the present study, and the area for entry into each trap was much larger (i.e. the entire perimeter) than is usually the case in minnow traps which are entered through small openings in either end. The larger opening should reduce the likelihood of exclusion of small crayfish by larger individuals as found by Malley and Reynolds (1979) and because large male Paranephrops zealandicus do not appear to be

particularly aggressive towards smaller conspecifics (suggested by my observations of crayfish of all sizes feeding together in traps placed in the shallows of Lake Georgina) this may not have been a problem anyway.

Other criticisms of trapping methods have centred mainly on the use of baits. Meat baits have been reported to catch larger crayfish (e.g. Cherax tenuimanus - Morrissey, 1972; Cambarus bartoni and Orconectes virilis- Somers and Stechey, 1986), and more crayfish per unit sampling effort than cereal baits (Somers and Stechey, 1986). The length of the sampling period also may bias a sample, as larger crayfish may, by virtue of relative size, move faster than smaller individuals and thus reach the bait before them (Morrissey, 1972). Therefore, larger individuals may be over-represented if the sampling period is too short.

The two samples that I took during each sampling period at each site were always similar in terms of mean size and size range of individuals present, although larger individuals were dominant in catches and large males were caught more frequently than large females. The dominance therefore may at least partly be the result of sampling bias induced by trap and bait effects. It also may reflect true population structure. Abrahamsson (1966) found that a population of Astacus astacus in ponds in southern Sweden was dominated by large crayfish. His information on population structure was gained by electric fishing - a method described as efficient by Malley and Reynolds (1979). He also reported male dominance in larger size classes as did Hopkins (1967), Hazlett and Rittschof (1985) and Lake and Sokal (1986) sampling different crayfish species using electric fishing techniques, hand

collection and box traps, respectively.

Crayfish smaller than 12 mm CL were not caught from Lake Georgina by the trapping method. However, four individuals which may have been young-of-the-year (5.3-6.5 mm CL, which is within the range of 0+ individuals of P. planifrons taken by Hopkins, 1967) were collected in mud samples from the Chara beds.

As soon as young Pacifastacus leniusculus leave the mother they are subject to predation from kin (Mason 1977). Similar predation on young Paranephrops zealandicus by other larger night-feeding crayfish may mean that small individuals remain in shelter (weed beds, under stones) while foraging and if so this could help explain their absence from traps.

The need to catch individuals in a wide range of sizes for analysis of size/site related diet and distribution trends was complicated by the problem of attracting crayfish from other sites. Before the regular sampling programme was initiated, sampling for various time periods and subsequent gut analyses suggested that a trapping period of one hour was long enough to ensure a reasonable catch but short enough to to reduce bias introduced by attraction of crayfish from other weeds beds. In addition, the traps were placed near the centre of each weed bed, at least 10 metres from each adjacent zone to minimise trapping of migrants.

Points and occurrence were both used in this study as recommended by Hyslop (1980) to distinguish between common and rare food species and to increase the accuracy of assessment of relative importance of prey types. Each method is biased in assessing the relative importance of foods containing different

proportions of soft and hard parts but this is unavoidable. Points, based on the volumetric contribution of each item in the gut favour hard materials because of the more rapid breakdown of soft tissues by the gastric mill. However, by correcting for gut fulness, points for a given food type will be dominated by scores from crayfish with full or nearly full guts (Williams, 1981). This procedure should reduce bias toward food items with longer digestion times, items which are more likely to be present in emptier guts. The occurrence method is similarly biased for the same reason. To help overcome this bias Williams (1981) suggested using only guts which were at least 50 %. full. I included guts which were 25 % full in my analyses but they accounted for only 6 % of all guts containing food. About half of these were from crayfish collected in July when very little hard material was present. Finally, by sampling during the peak feeding period (night) the chance of finding freshly ingested material was maximised, and biases introduced by differential digestion should have been reduced.

2.5.2 Diet

The crayfish in Lake Georgina consume most available food materials. They display an opportunistic strategy similar to that reported for several other species (Gaeveskaya, 1966, Momot et al., 1978; Lorman and Magnusson, 1978). Fluctuations in diet usually corresponded to changes in food production (i.e. abundance

of epilithon) and in energetic requirements (i.e. moulting and breeding).

As opportunists, freshwater crayfish generally consume what is available at any one time. Thus plant material may have dominated the diets in populations of several well-studied species (e.g. Abrahamsson, 1966; Gaeveskaya, 1966; Momot et al., 1978; Lodge and Lorman, 1987; present study) because fresh or decayed allochthonous and/or autochthonous plant material provided the most readily available food sources.

The ratio of plant to animal material in the diet also may vary with crayfish size and season. Juvenile crayfish generally are considered more omnivorous than adults. Abrahamsson (1966) suggested that a reduction in animal biomass in the diet with increasing crayfish size may be the result of a reduction in dexterity. Smaller crayfish, he suggested, are faster and more precise in their movements and thus able to catch swifter prey (e.g. cladocerans and copepods). Thus, they may use a wider range of foods than adults. However, adults may need to consume fresh animal tissue during periods of increased energy requirement (i.e. moulting and breeding; Gaeveskaya, 1966) but the target animals are generally slower moving species (e.g. molluscs; Abrahamsson, 1966).

Large populations of benthic invertebrates are lacking in Lake Georgina. The gastropods Potamopyrgus antipodarum, Physa acuta, and Gyraulus corinna, have been reported from weed beds but not the benthos, and rarely appeared in guts (and are likely to have been dead upon ingestion). Insects and fish were also included in the diet to a limited extent (but again are likely to have been

dead upon ingestion) and were consumed equally by crayfish of all sizes. It is possible that the low availability of animal tissue is balanced by seasonally abundant epilithon, of high food quality.

The Carbon:Nitrogen ratio of epilithic material (= Aufwuchs; McMahon et al., 1974) varies with turnover rate and species composition. McMahon et al. suggested that the predominant Aufwuchs communities of lakes were characterised by low C:N ratios and high biomass turnover, and were likely to be dominated by diatoms, blue-green algae and bacteria as in Lake Georgina. They also considered that the high nitrogen levels typically found in such communities were indicative of high protein concentrations and therefore high nutritive value to herbivores. The epilithic community in Lake Georgina therefore is likely to be a highly nutritive, seasonally abundant food source, a condition which could account for its importance in the diet of the crayfish during spring and summer.

The temporal consumption of exoskeletal material seems to be linked with moulting periodicity. Gastrolith production (an indicator of moulting) peaked during summer and early autumn and presumably was accompanied by an increase in availability of exuviae in the benthos. The increase in dietary exoskeletal material in March seems to reflect this moulting peak. By May, the number of crayfish moulting was lower as was the incidence and abundance of exoskeletal material in the diet. Both moulting and dietary exoskeleton increased again in September and November.

As discussed previously, the abundance of plant material in the diet appears to reflect its availability. However, many

animal tissues are more readily assimilable than plant tissues and are potentially superior foods (Moshiri and Goldman, 1969). Whether that potential can be realised is dependent on food availability, which dictates its real energetic worth. If animal tissue is common then switching to it from a plant dominated diet can be advantageous. Such switching behaviour has been reported for Astacus astacus in response to increased energy requirements associated with breeding and moulting (Gaeveskaya, 1966 , Jarvekugl 1958 - in Gaeveskaya, 1966). During the energetically expensive juvenile phase, A. astacus (Gaeveskaya, 1966) and Orconectes virilis (Momot et al., 1978) also may consume more animal tissue than they do in adult life but this was observed in situations where animal prey appeared to be more abundant than in the present study. The relative paucity of animal prey, in Lake Georgina, indicated by its generally low incidence in the diet of the crayfish (present study), and by a recent survey undertaken by Glenny et al. (1987) suggest that there is little advantage to be gained by switching and therefore other abundant but probably less energy rich materials continue to be relied upon as food.

2.5.3 Distribution

Distributional patterns observed during this investigation were similar to those reported in other studies of the same (Quilter 1975, Devcich, 1979) and different (Abrahamsson and Goldman 1970, Flint, 1977) genera of Decapoda. Similarities arise

from common effects of the same abiotic (i.e. temperature and light) and biotic (i.e. moulting and breeding) variables. Dissimilarities arise from the expression of these variables as determined by the conditions of substrate, cover and food availability peculiar to the habitat under study.

The crayfish in Lake Georgina are confined to the three vegetation zones detailed previously (Chapter 1) and few animals were seen in the spring (i.e. below about 5 m) during night or day when observations were made with SCUBA. The spring is steep sided and contains unstable fine sediment similar to that reported to occur at the lower depth limit of P. planifrons in Lake Rotoiti (North Island) (Devcich 1979). Movement may be difficult in such substrata and crayfish in Lakes Rotoiti and Georgina have been observed sinking into the bottom, leaving deep tracks.

Periods of increased activity in autumn and spring, appeared to coincide with periods of increase in energy requirements. The onset of torpor during winter (Tack, 1941; Momot 1967; Quilter, 1975) may necessitate a build up of energy reserves during autumn (Armitage, 1972; Devcich, 1979). Devcich reported an autumn build-up of hepatopancreatic lipid and energy in spring-breeding, female P. planifrons in Lake Rotoiti. As winter temperatures in Lake Rotoiti did not drop below 10°C, Devcich suggested that the need for all members of the population to increase their energy stores may have been reduced. However, an increase in stored energy may be required by spring-breeding females whose eggs develop in winter. In Lake Georgina, males, and gravid and non-gravid females all increased feeding activity in May, presumably in anticipation of a possible period of torpor during

winter when temperatures are much lower than those experienced by the Lake Rotoiti population.

Feeding activity increased again in September and November, when the water temperature rose and gastroliths appeared suggesting the prescence of a spring moult. Such a moult has been reported in P. planifrons (Hopkins, 1966), Orconectes virilis (Momot and Gowing, 1977) and O. kentuckiensis (Boyd and Page, 1978). The higher water temperature also may enhance the rate of egg development and therefore female energetic requirements and account for the increased level of female activity in both September and November.

As an opportunistic omnivore, P. zealandicus should feed on whatever food is available in the area it occupies during the feeding period. If adequate shelter is present in the foraging area it may not be necessary to seek cover elsewhere during daylight hours. However, migrations (directed movements) may still occur if inadequate shelter is available for some members of the population (i.e. limited by size or number). Devcich (1979) suggested that a diel migration of most adult P. planifrons between shallow water feeding areas and the aphotic zone of Lake Rotoiti resulted from a shortage of suitable sized shelters in the littoral region. Such a limitation did not apply to juveniles which were able to forage and shelter in the shallows. The development of a size specific, nocturnal feeding strategy, the periodicity of which is determined by a well developed circadian rhythm based on a negative phototactic response (Quilter, 1975) could be the result of predation pressure. In any water body, the intensity of light reaching the bottom in the shallows is greater

than that reaching deeper areas. Therefore, the inhibitory effect of light will vary with depth and seasonal shifts in specific wavelengths of light reaching different areas will result in some variation in degree of inhibition (Devcich 1979).

In Lake Rotoiti, the evening migration to the shallows begins with activity in deeper areas, and crayfish move progressively into shallower areas as light levels there become non-inhibitory (Devcich 1979).

Shelter in the western shallows of Lake Georgina also appears to be limited by the generally small size of substrata - largely cobbles with interstices filled with an algal turf. Movement into the shallows from other areas during feeding periods is suggested by the presence of Chara and Elodea in gastric mills of crayfish caught in the area. However, such movements may not be migrations of the kind reported by Devcich, as the relative distribution of food and shelter within the area occupied by crayfish is somewhat different from that in Lake Rotoiti. In particular, food and shelter appear to be closely associated in both Chara and Elodea beds. The eastern shallows, on the other hand appear to offer more shelter and less food than their western equivalent. Occupation of the shallows (especially those in the west) therefore may not result from migration but occur through less directed movements of crayfish as feeding activity progresses. Such movements may result in dispersal from intermediate to deep sites and vice versa as suggested by the presence of Chara and Elodea in the guts of crayfish taken from these areas.

Temperature also affects activity levels (Somers and Stechey, 1986), growth (Devcich, 1979) and the reproductive cycles of

crayfish (Berrill and Arsenault, 1982), and members of some species seek shelter as temperatures drop (Tack, 1941; Flint, 1977). Movement into deeper water was evident in Lake Georgina in May and July when no crayfish were caught at the shallow western site. Similar offshore movements have been reported by Tack (1941) and Flint (1977) for Cambarus immunis and Pacifastacus leniusculus, respectively.

Finally, gross changes in the abundance and quality of food appear to have influenced the distributions of crayfish in Lake Georgina. The western shallows support an epilithic and epibenthic algal community whose biomass peaked in summer, and provided a rich source of food. Algal growth presumably was optimised by high water temperatures and the long summer photoperiod. The shallow slope and small rock and cobble substrata with accessible epilithon may account for the greater crayfish catch in this western area compared to that at the corresponding site on the eastern transect. In the latter part of the lake, the presence of larger rocks may result in reduced accessibility to food thereby reducing the effective food value of the area. The presence of a higher proportion of inorganic sediments in guts of crayfish taken from the eastern transect also supports the idea of poorer food quality there.

Availability of ingestible organic material is unlikely to have a direct influence on the distribution of crayfish in Elodea and Chara beds, but the weed beds differ greatly in structure and Chara beds with their tightly packed mass of plants, may provide more shelter particularly during winter.

To summarize, the P. zealandicus population in Lake Georgina displays an opportunistic, omnivorous feeding strategy typical of crayfish in general (Momot et al., 1978). Diet appears to be governed by the relative abundance of available foods and animal tissues were not selected preferentially by juveniles or breeding and/or moulting adults as described for some other species. Temperature appears to be a major variable governing the seasonal activity of crayfish as it is in other high altitude, high latitude populations (Aiken, 1968; Momot, 1984).

CHAPTER 3

DIGESTION AND THE ROLE OF MICROBIAL ENZYMES

DIGESTION AND THE ROLE OF MICROBIAL ENZYMES

3.1 Introduction

Many freshwater crayfish are opportunistic omnivores that consume a wide variety of fresh and decaying plant and animal tissue (Momot, Gowing and Jones, 1978). Even though physical breakdown of most ingested material is efficient, its utilisation is limited by the enzymatic reservoir available for digestion. In particular, an inability to breakdown structural polysaccharides is widespread among freshwater invertebrates (Bjarnov, 1972; Monk, 1976, 1977; Martin et al.,1980, 1981a, 1981b; Barlocher and Porter, 1986) and Telford (1970) found no cellulolytic activity in the crayfish Orconectes virilis (Hagen) or O. propinquus (Girard). Carboxymethylcellulose can be digested by both Astacus fluviatilis Fabricius (Kooiman, 1964) and Procambarus clarkii (Girard) (Yokoe and Yasumasu, 1964) but activity towards native celluloses remains untested.

In the absence of host-specific cellulases, reliance must be placed on ingested microbial enzymes and /or microbial "conditioning" of food if any use is to be made of otherwise refractory plant material. Furthermore, such conditioning must proceed to the point where host-specific enzymes can digest at least some of the ingested material. The low levels of cellulolytic activity reported in the amphipods Gammarus pulex L.,

G. lacustris Sars and G. tigrinus Sexton (Monk, 1976; Barlocher and Porter, 1986) may be of microbial and/or host-specific origin, but that found in freshwater insects (11 of 42 species examined in the aforementioned studies) is thought to be primarily of microbial origin.

The role of ingested microbial enzymes has been investigated in several freshwater invertebrates. Barlocher (1982) found that Gammarus fossarum Koch benefitted from ingested fungal enzymes. G. tigrinus may also benefit in this way (Barlocher and Porter, 1986) and according to Sinsabaugh, Benfield and Linkens (1981) up to 50% of the cellulase activity observed in the midguts of larval Allonarcys proteus (Newman) (Plecoptera) was acquired from ingested micro-organisms. In contrast, Chamier and Willoughby (1986) were unable to demonstrate any cellulolytic activity in G. pulex that could be attributed to ingested fungal enzymes, but suggested that exogenous fungal hydrolysis assisted host-specific breakdown of cellulose.

In this chapter carbohydrase and protease activity in the crayfish is considered and the roles of microbial enzymes in the digestive process are assessed.

3.2 Materials and Methods

3.2.1 Gut Enzyme Assays

Prior to their use in experiments, crayfish were kept in laboratory aquaria and fed detritus collected from Lake Georgina. Crayfish were killed by freezing at -80^{o}C for 4 - 5 minutes, their hepatopancreases were removed, placed in a glass vial and macerated in cold, sterile distilled water (30 ml). After centrifugation (10000 g, 4^{o}C, 20 min) supernatant was filtered to remove remaining solids (Whatman GF/A on Millipore apparatus) and used immediately for enzyme assays. Pooled extracts from 10 animals (10 - 12 g fresh weight) were used in each series of assays.

3.2.1 Substrates

Nine substrates were selected as representative of the dietary range of crayfish. They were microcrystalline cellulose (MCC), carboxymethyl cellulose (CMC), cellobiose, amylose, pectin, mannan, laminarin, chitin and 'Azocoll'(a dye - collagen complex). MCC (native cellulose powder), CMC (a soluble derivative of cellulose) and cellobiose (a breakdown product of cellulose) are representative of the structural polysaccharides of higher plants. Citrus pectin (D - galacturonic acid) is found dissolved in plant juices and its insoluble form, protopectin, occurs as a structural polysaccharide complexed with cellulose (Berk, 1986). Alpha

amylose (alpha-1,4, glucan) is the storage polysaccharide in all chlorophyll-containing plants. Laminarin was chosen to represent the beta-1,3 glucans found in diatoms (as chrysolaminarin - a storage sugar, Werner, 1977) and complexed with chitin in fungal cell walls. Chitin (N-acetyl glucoseamine polymer) also occurs in crustacean exoskeletons (the form used was complexed with an azure dye) and mannan is a storage sugar in fungi (Burnett and Trinci, 1979). Collagen was selected as a representative animal protein .

3.2.3 Assays

Activity towards all substrates except chitin and Azocoll was determined by measuring the amounts of reducing sugars produced in a standard assay (Bernfield, 1955; Trought, 1982). The amount of dye released provided a measure of activity towards chitin and collagen (Barlocher and Porter, 1986). Buffers used in all assays were 0.1M $C_6 H_8O_7 . H_2O$ / 0.1M Na_2HPO_4. Exactly 0.5 ml buffered substrate (2% w:v, 5% w:v - chitin and MCC or 0.5 ml buffer + 15 mg - Azocoll) was mixed with 0.5 ml of extract. Two drops of toluene were added to half the preparations (5 replicates) to prevent microbial activity during incubation although it need not preclude the activity of microbial enzymes already present in the extract. No toluene was added to the other preparations so that the contribution of microbial enzymes to substrate digestion could be assessed. Sterile techniques, including filtration of soluble substrates (Advantec, 0.2 m.) were used throughout. Where filtration was not possible (when using MCC or Azocoll as the substrate) contamination was checked by incubating substrate and

buffer without extract.

The effectiveness of toluene as an inhibitor of bacterial enzyme activity was examined by comparing the results of assays incorporating toluene with those obtained with other chemical inhibitors and physical treatment. A series of 35 assays was run using the method described previously. The series comprised seven treatments (**Table 3.1**), each with five replicates containing gut extract, buffer and Azocoll. Azocoll was used because significant host-specific enzyme activity and apparent microbial activity had been demonstrated with standard assays (refer section 3.3.2 - results). Preparations were treated physically with ultrasound to determine the potential effect of bacterial cell lysis on enzyme activity. Such cellulytic properties were attributed to toluene by an anonomous reviewer of an earlier manuscript.

After incubation in the dark for 4 hours at 15^{o}C all reactions except those with chitin and Azocoll were terminated by adding 1 ml dinitrosalicylic acid reagent (DNS) and heating at 90^{o}C for 5 minutes. After addition of 5 ml distilled water and cooling for 5 minutes, absorbance was measured at 540 nm (Kontron Uvikon 860 spectrophotometer).

Prior to addition of DNS to MCC assays the mixture was filtered (Whatman GF/C) to remove insoluble substrate. Chitinase activity was terminated by filtration and absorbance was measured at 575 nm. Protease activity in the presence of Azocoll was terminated by adding 3 ml. 10^{-3} M HCl before filtration (Martin et al., 1980) and absorbance was measured at 520 nm. Aliquots of boiled extract (three replicates per treatment) were used as controls for all assays.

Table 3.1 Treatments used in testing the effectiveness of toluene as an antimicrobial agent.

Treatment	Action	Dosage
A. Chemical Agents		
1. Benzylpenicillin + Streptomycin sulphate	antibacterial	30 g/ml buffer
2. Mycostatin (Nystatin B.P.) + Actidione	antifungal	50 g/ml buffer
3. 1. + 2.	both above	
4. Toluene	antimicrobial	2 drops per tube.
B. Physical Agents		
5. Ultrasound (Mettler Electronics Ultrasound Cleaner)	cell lysis	30 secs per tube.

Antibiotics were added to aliquots of buffer prior to addition to the remaining components of the appropriate assay. Two control treatments were prepared, one without any additives (i.e. with extract, substrate and buffer), the other similar but containing aliquots of boiled extract .

Results of chitinase and protease assays were expressed as 'units of activity' per ml extract / h. Reducing sugar production in all other assays is given as maltose equivalents (obtained by reference to a standard curve, **Fig 3.1**). 3.2.4 Foregut pH

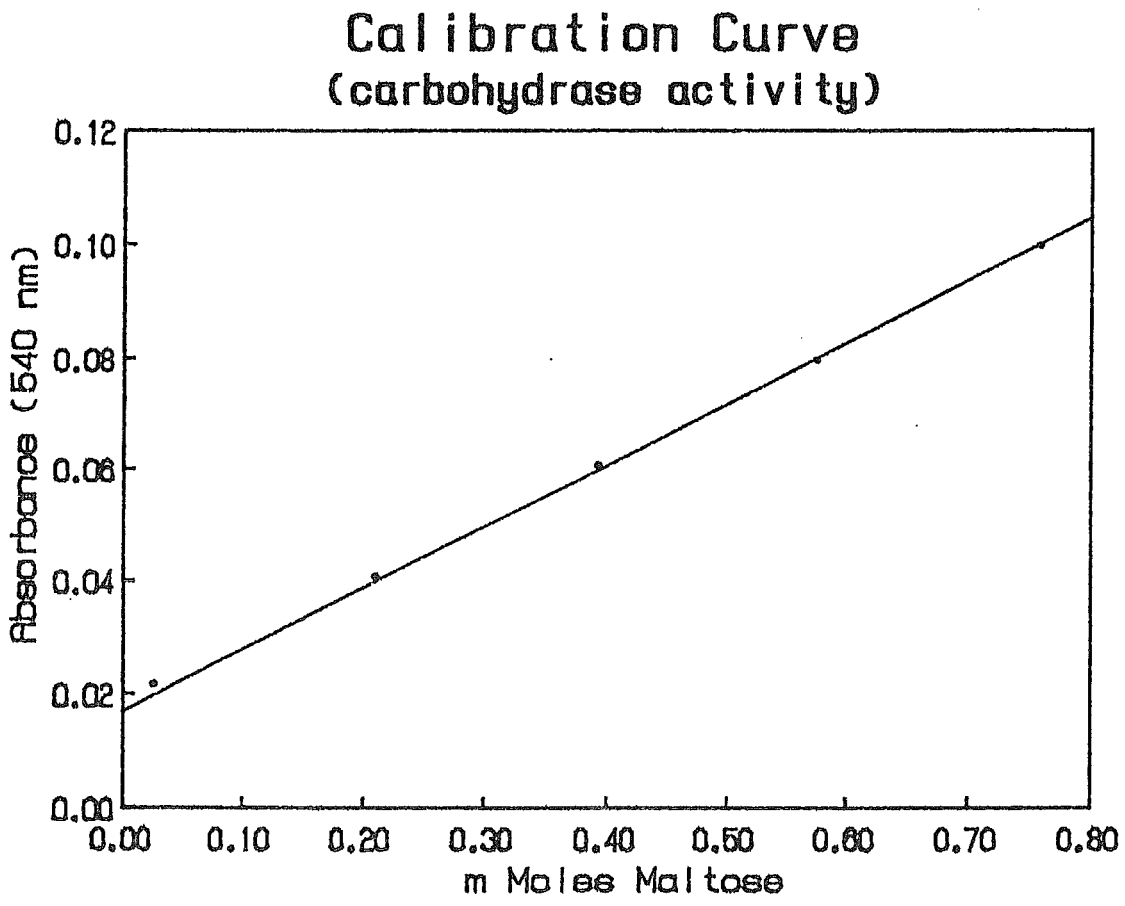
Foregut (gastric mill) pH was used as an indicator of hepatopancreas pH as the digestive juice of the foregut originates in the latter organ (Gibson and Barker, 1979). An isoelectric focussing electrode (Solstat EPM 300) was inserted into the guts of 20 freshly caught animals, and the mean pH value obtained (5.5 $\pm$ 0.3 , 95 % C.L.) was used when setting the pH of the buffer used in enzyme assays. Gut pH, checked using close range indicator paper before each assay, never exceeded the 95% confidence interval stated above.

3.2.5 Detrital enzymes and bacteria

Fresh Elodea from Lake Georgina was decayed in aerated lake water at 15 - 20^{o}C and after 44 days it closely resembled the "detritus" typically found in crayfish guts. At that time, samples (500 mg wet weight) were macerated in cold buffer (0.1 N $NaHCO_3$ / Na_2CO_3) and adjusted to either pH 8.7 or 6.6, the extreme pH values recorded in the lake.

After centrifugation (4000 r.p.m., 20 min), the supernatant was incubated in the dark for 20 h at 15^{o}C (5 replicates and 3 substrate controls) and tested for reducing sugar activity using MCC and laminarin as substrates. Bacteria were isolated from the decayed Elodea by inoculating loopsful of macerate (Elodea in

Fig 3.1 Calibration curve used in carbohydrase assays.
mMoles maltose = - 0.155 + 9.1165 (absorbance)


Calibration Curve
(carbohydrase activity)
Absorbance (540 nm)
0.12
0.10
0.08
0.06
0.04
0.02
0.00
0.00
0.10
0.20
0.30
0.40
0.50
0.60
0.70
0.80
m Moles Maltose

sterile distilled water) on to nutrient agar plates. Plates inoculated with water alone served as controls. After incubation for 14 days at 20°C bacterial colonies were Gram stained and viewed at 1000 X magnification using oil immersion. Further isolation and identification of bacteria were carried out by staff of the Pathology Department, Christchurch Hospital (see Winterbourn, 1982 for details). Fragments of decayed Elodea were examined by scanning electron microscopy (SEM) after preparation using standard techniques (Rounick and Winterbourn, 1983).

3.2.7 Gut Microflora

Interior surfaces of gastric mills, inner and outer surfaces of hepatopancreas tubules, digestive juices and hepatopancreas extracts were examined for bacteria. Hepatopancreases from five freshly killed animals were removed, pooled and homogenised in 100 ml sterile distilled water. After 1:10 dilution, samples were :

a) Placed on glass slides, Gram stained , and viewed at 1000 X magnification with an oil immersion lens.
b) Drawn on to Nucleopore filters (0.2 µm), fixed in buffered (0.12 M Na Cacodylate, pH 7.2) 2.5% gluteraldehyde for 2 h and dehydrated in an alcohol series. After drying, filters were mounted on stubs, coated with gold and viewed by SEM at magnifications up to 5000 X.
c) Inoculated on to nutrient agar plates which were incubated at 20° C for 14 days. Further isolation and identification of bacteria were again carried out by staff of the Pathology

Department, Christchurch Hospital. The isolated cultures were inoculated into aliquots of Purple Broth Medium (Cowan, 1979) containing MCC (5% w:v) or laminarin (2% w:v) (selection of substrata was based on results of carbohydrase assays, section 3.3.1). Samples of digestive juice from the gastric mills of five crayfish were examined in the same three ways and organ surfaces were examined by SEM. The latter were prepared as described above except that after alcohol dehydration they were run through an amyl acetate series and critical point dried before coating with gold.

3.3 Results

3.3.1 Carbohydrase Activity

Hepatopancreas extracts showed activity toward all eight test substrates (Table 3.2). Activity was greatest with amylose, chitin, pectin and laminarin but the absolute figures should be treated with caution as they represent single substrate hydrolyses under ideal conditions. Digestion in vivo may be somewhat different because of the greater complexity of natural substrata. Statistically significant differences in activity between toluene-added and toluene-absent assays were found for MCC and laminarin (t-test, $P < 0.05$). Activity was not detected in toluene-added assays with MCC as substrate and implies that

microbial enzymes alone were responsible for activity measured in the toluene -absent assays.

3.3.2 Protease activity

Activity towards Azocoll was pronounced (**Table 3.2**) and significantly higher in the toluene-free assays (t-test, $P < 0.05$). This indicates that collagen is hydrolysed by host-specific enzymes and those derived from micro-organisms present in the gut.

3.3.3 Gut Microflora

Three genera of Enterobacteriaceae (*Hafnia*, *Citrobacter* and *Aeromonas*) dominated the microflora of both the digestive juices and the hepatopancreas. Members of all three genera are found commonly in water, soil and the alimentary canals of a wide variety of animals (Krieg and Holt, 1984). Resident micro-organisms were not found on the inner walls of the gastric mill or on the outside of the hepatopancreatic tubules (**Fig 3.2**) which suggests that microbial enzyme activity detected in assays using pancreatic tissue was due to populations within the tubules. Neither MCC nor laminarin were degraded by the isolated cultures in the Purple Broth Media.

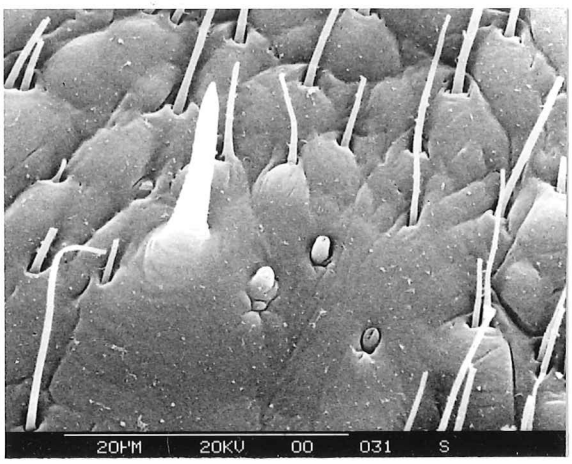
Table 3.2 Carbohydrase, chitinase and protease activity (x ± 95 % CL, n=10) obtained in assays at 15°C with extracts of *P. zealandicus* digestive gland and 9 substrates. P values refer to comparisons between toluene - added and toluene - lacking assays using Students t . ND - none detected.

Substrate	Toluene added	Without toluene	t	P
A) Carbohydrase activity (mM maltose (ml extract)$^{-1}$h^{-1}				
MCC	ND	0.089 (0.083)	-	-
CMC	0.385 (0.369)	0.335 (0.229)	-	N.S.
Cellobiose	0.304 (0.287)	0.269 (0.225)	-	N.S.
Amylose	0.843 (0.301)	0.799 (0.462)	-	N.S.
Pectin	0.442 (0.195)	0.691 (0.072)	-	N.S.
Mannan	0.334 (0.192)	0.399 (0.172)	-	N.S.
Laminarin	0.231 (0.141)	0.597 (0.130)	10.942	0.05
B) Protease and chitinase activity (activity (ml extract)$^{-1}$ h^{-1})				
'Azocoll'	0.489 (0.157)	0.964 (0.236)	7.343	0.05
Chitin	0.704 (0.329)	0.695 (0.576)	-	N.S.

Fig 3.2 Scanning electron micrographs of the gastric mill and the hepatopancreas of Paranephrops zealandicus.

Top and middle : Close-ups of the interior of the gastric mill (scale bar = 20 um) and the surface of one hepatopancreatic tubule (scale bar = 40 um). Bacteria were absent from both surfaces.

Bottom: Cut end of an hepatopancreatic tubule showing glandular interior. Scale bar = 40 um.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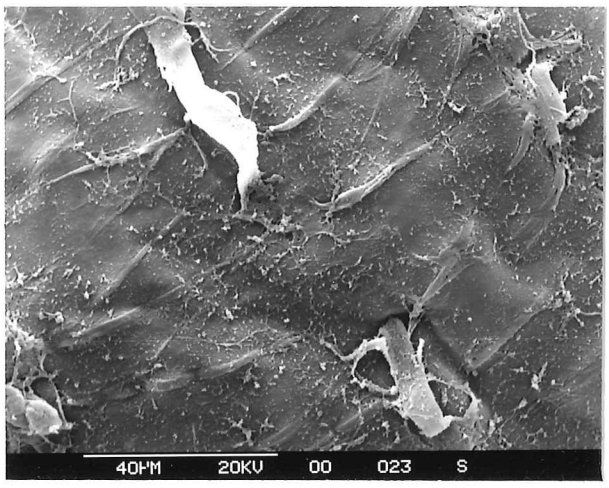
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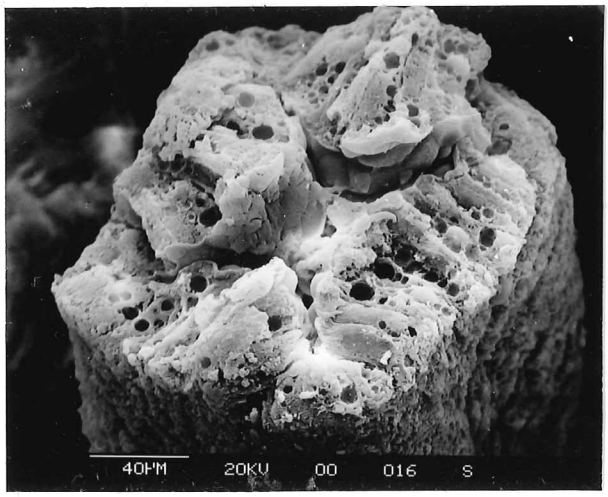
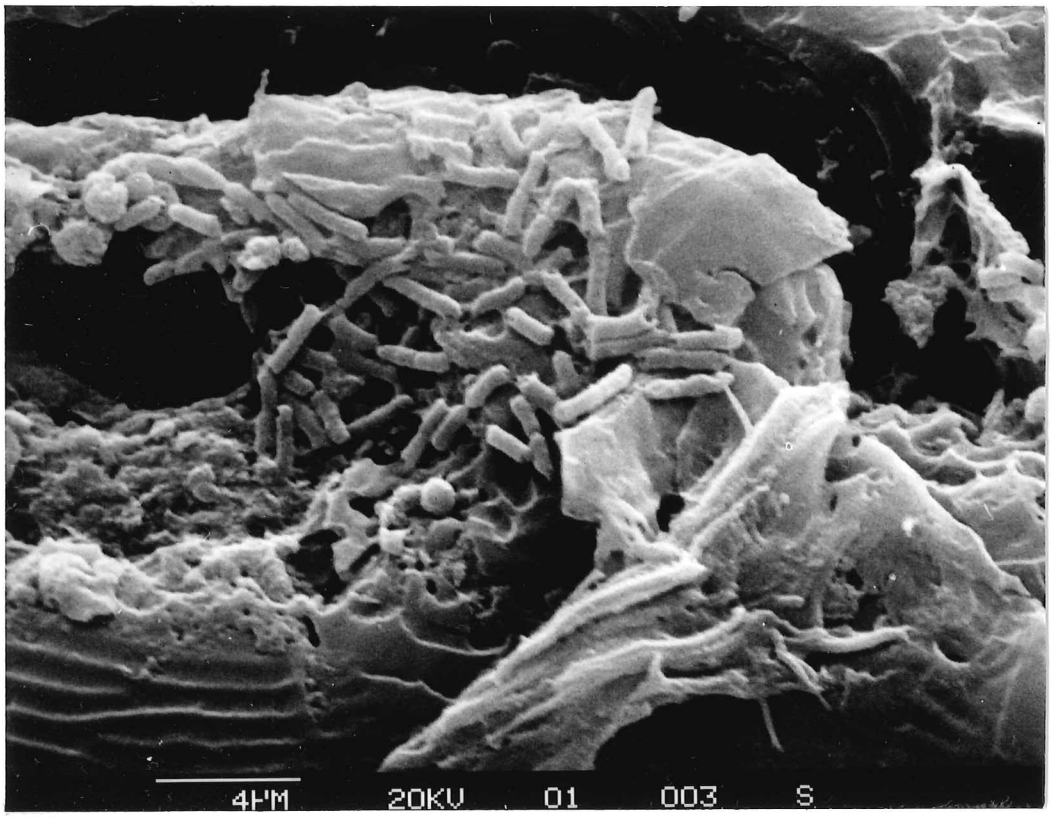

40PM 20KV 00 016 S

Fig 3.2 contd. Scanning electron micrograph of an hepatopancreatic fragment on a Nucleopore filter. Note rod-shaped bacteria. Scale bar = 4 um.


4PM
20KV
01
003
S

3.3.4 Detrital assays and microflora

Low enzyme activity towards laminarin and MCC was detected in solutions prepared from Elodea which had been allowed to decompose for 44 days (**Table 3.3**). Extensive bacterial and fungal populations were present on the decayed material (**Fig 3.3**) and the former covered most of the visible surface. Of the three bacterial genera isolated from the alimentary canal, only Citrobacter (two strains) was isolated from plates inoculated with detrital suspension although Gram staining also revealed very small populations of cocci and Gram positive rods.

3.3.5 Toluene

All experimental treatments showed reductions in catalytic activity below that of the 'no-additive' control (**Table 3.4**). Significant reductions (Sudents t, $P < 0.05$) were shown by treatments 3 (four antibiotics) and 4 (toluene). Toluene inhibited activity to a significantly greater degree than all other treatments (Students t, $P < 0.05$) .

Table 3.3 Enzyme activity (mmol maltose / ml extract/h ; x ± 95% CL) recorded in 15°C assays with decaying Elodea extract and MCC and laminarin as substrates. ND = none detected.

Substrate	pH	
	6.6	8.7
MCC	0.009 (0.006)	ND
Laminarin	0.033 (0.011)	0.031 (0.025)

Fig 3.3 Scanning electron micrograph of a section of Elodea leaf, showing bacteria, fungal hyphae and a diatom, after 44 days decomposition. Scale bar = 4 um.

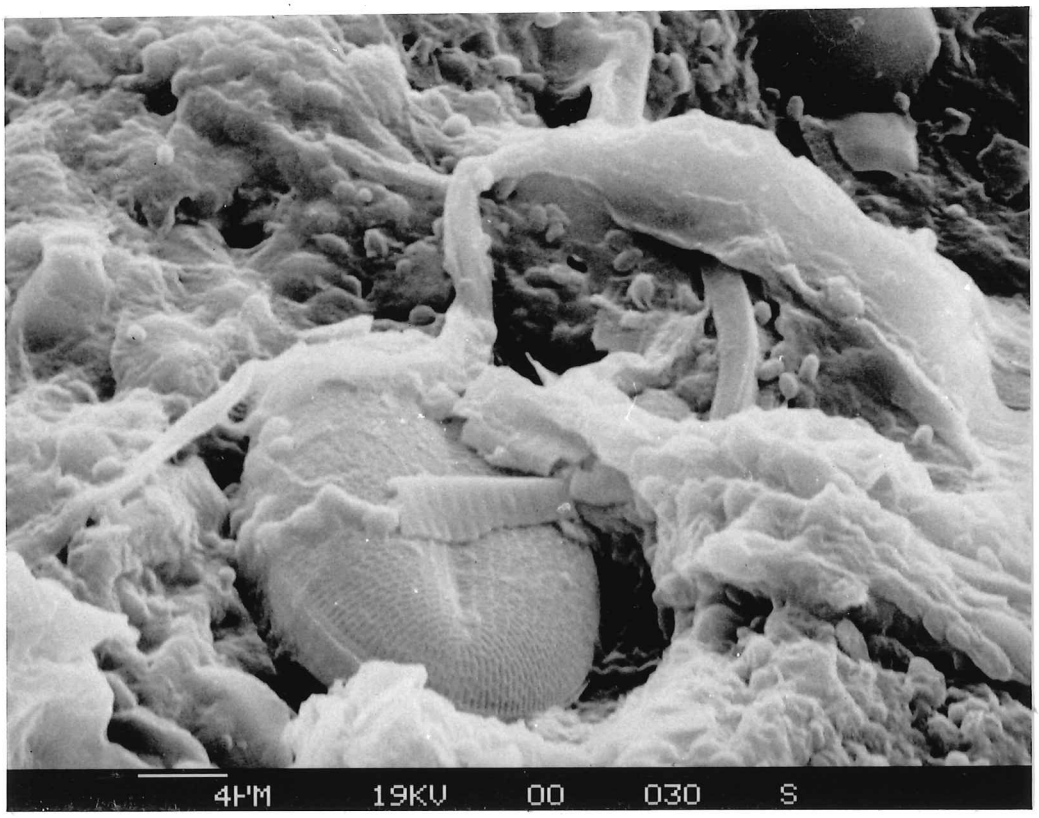

4µM
19KV
00
030
S

Table 3.4 The effectiveness of toluene as an antimicrobial agent. Measured as protease activity (activity (ml extract)$^{-1}$ h^{-1}, mean ± 95% CL, n=5) obtained in assays at 15°C with extracts of *P. zealandicus* digestive gland with 5 experimental and two control treatments.

Treatment	Protease Activity
A. Chemical Agents	
1. Benzylpenicillin + Streptomycin sulphate	0.768 (0.031)
2. Mycostatin (Nystatin B.P.) + Actidione	0.784 (0.042)
3. 1. + 2.	0.747 (0.016)
4. Toluene	0.648 (0.061)
B. Physical Agent	
5. Ultrasound (Mettler Electronics Ultrasound Cleaner)	0.764 (0.023)
C. Control	
6. No additives (extract, substrate and buffer)	0.800 (0.031)

Note: The boiled-extract control was used as a zero when determining activity.

Significant comparisons ($t_{tab\ 0.05(df=8)} = 1.860$)

	t	P
3. and 6.	2.950	0.05
4. and 6.	4.330	0.05
4. and 1.	3.207	0.05
4. and 2.	3.577	0.05
4. and 3.	3.064	0.05
4. and 5.	3.515	0.05

3.4 Discussion

3.4.1 Method

Although toluene has been used extensively in invertebrate digestive-enzyme studies to inhibit microbial activity (e.g. Bjarnov, 1972; Kristensen, 1972; Monk, 1976, 1977; Winterbourn, 1982; Barlocher and Porter, 1986; Chamier and Willoughby, 1986) few studies have tested the effectiveness of the chemical itself. The only traceable comparative work (Winterbourn, 1982) obtained similar results with both toluene and antibiotics when testing cellulolytic activity in caddisflies. The present study has produced comparable results and confirms that the addition of toluene provides an adequate method of microbial control in such studies.

3.4.2 General

Crayfish are opportunistic feeders (Lodge and Lorman, 1987) and like Orconectes virilis (Momot et al., 1978), Paranephrops zealandicus feeds on detritus and living aquatic macrophytes. O.propinquus also feeds on living higher plants (Lorman, 1980) yet neither it nor the others have endogenous enzymes that are able to degrade native celluloses (Telford, 1970; present study). However, a low level of cellulase activity of microbial origin was demonstrated in gut extracts from P. zealandicus and it may be of some value to the crayfish. For example, it may have a 'tool

function' (Kristensen, 1972) solubilising celluloses to the extent that they and their derivatives can be attacked by host-specific enzymes. The tool function is an extension of the conditioning that takes place in the detrital pool, and micro-organisms involved in the latter may continue to "condition" ingested material in the gastric environment .

For cellulose digestion to be most efficient under any conditions a complex of endo- and exoenzymes must be present. The endoenzymes fragment cellulose molecules and in so doing expose points of attack for exoenzymes. The latter hydrolyse bonds near the non - reducing end of the polymer to produce soluble oligosaccharides, cellobiose or glucose (Reese, 1977; Eriksson, 1978).

Assays with CMC as substrate test for the presence of exo B - 1,4 glucanase activity (Leisola et al., 1975) and by inference the prior activity of endo B - 1,4 glucanases since the former cannot function efficiently without the latter. Endoglucanase activity was not investigated in the present study but the ability to make use of partially hydrolysed forms of cellulose was apparent as relatively high, although variable activity was recorded in the CMC and cellobiose assays. As preparations with and without toluene produced similar results, it appears that the enzymal contribution from microbes present in the hepatopancreas was not significant. Similarly, it can be concluded that amylose, pectin, chitin and mannan were broken down primarily and perhaps exclusively by host-specific enzymes.

Highest activities recorded with P. zealandicus extracts were for amylases even though 1,4 glucans (amyloses) are not

significant components of most decaying plant material (Martin et al., 1981b). However, they do occur in fungi and filamentous algae which are ingested by crayfish along with detritus. Also, some amylases can break down glycogen (an alpha 1,4 and 1,6 linked glucan) which is likely to be present in animal detritus (Kristensen, 1972; Gibson and Barker, 1979) although, as Wyatt (1967) points out, an additional enzyme is needed for glucose production.

A significant increase in enzyme activity in the absence of toluene was recorded only when collagen and laminarin were provided as substrates. This implies a role for micro-organisms in the digestion of animal flesh, diatoms and fungal hyphae, although I could not substantiate this hypothesis with laboratory cultures.

In summary, it appears that P. zealandicus is not able to use structural polysaccharides such as cellulose without some form of prior conditioning mediated through microbial activity. Such an inability is widespread among marine (Kristensen, 1972); freshwater (Monk, 1976) and terrestrial (Nielson, 1962) invertebrates, although the ability to digest storage sugars and degraded forms of cellulose has been reported widely, especially in crustaceans (Yokoe, 1960; Yokoe and Yasumasu, 1964; Telford, 1970; Kristensen, 1972; Monk, 1976).

Paranephrops species occur in a wide range of lentic and lotic habitats and usually are described as feeding generalists (Chapman and Lewis, 1976; Devcich, 1979). As such they ingest a wide range of living and detrital plant and animal materials of aquatic and terrestrial origin. The enzyme reservoir of P. zealandicus

reflects their lack of specialisation, including as it does enzymes that degrade cellulose derivatives, tissues and storage sugars of diatoms, fungi, higher plants and animals.

CHAPTER 4

ASSIMILATION EFFICIENCY

ASSIMILATION EFFICIENCY

4.1 Introduction

The dominant environmental causes of variability in assimilation efficiency and growth of animals are temperature and food quality and quantity. In the case of crayfish variations in these factors may be reflected in moult frequency and increment, and the timing of breeding season. Variation may also be caused by different metabolic requirements of individuals of different size or sex (Somers and Stechey 1986).

Temperature also may effect endogenous digestive enzyme activity by modifying enzyme-substrate affinities or enzyme concentration (Hochachka & Somero, 1968; Behrisch, 1971; Newell, 1973). A decrease in temperature may result in increased enzyme activity, with compensation for otherwise lowered catalysis to the extent that the latter becomes independent of temperature (Somero and Hochachka, 1969).

During winter, the cessation of growth (Momot, 1984; France, 1985) and breeding (Berrill & Arsenault, 1982) and the onset of torpor (Brewis and Bowler, 1983) imply reduced metabolic requirements. Crayfish may remain active during periods of low temperature (Somers and Stechey, 1986), the extent of activity being limited by the volume of food ingested and its quality. At low temperatures activity may be facilitated by an increase in

assimilation efficiency.

Specific metabolic rates of crustaceans normally decline with increasing size, and as the moult frequency declines. Moshiri and Goldman (1981) found that the high moult frequency of early stages of Pacifastacus leniusculus and related high energy demands were compensated for by increasing ingestion and assimilation efficiency. Similar results were reported for Mitopus morio (Phalangida) by Phillipson (1960).

In this chapter the effects of temperature, food quality and crayfish size on assimilation efficiency are examined. The influence of temperature on enzyme activity is also considered.

4.2 Methods

4.2.1 Assimilation

Elodea canadensis was chosen as the food to be used in feeding experiments because it is the most abundant material ingested by crayfish in Lake Georgina.

Fresh Elodea collected from the lake was left to decay in aerated lake water for 1 month at 15 ^{o}C. Leaves were then removed from stems and dried to constant weight either immediately or after a further two weeks at 20^{o}C. All the resulting detritus was dried to constant weight and stored in a desiccator together with dried but un-decayed Elodea leaves. Two size classes of crayfish

(10 - 12 g (size class 2 - refer chapter 2) and 38-45 g fresh wt (class 4); M:F 1:1) were assigned randomly to individual 2 litre ice-cream containers of aerated tapwater maintained at various temperatures (**Table 4.1**) under a 12 h L:12 h D cycle and fed ad libitum with detritus from the lake. Females were used in the experiment only after the external condition of the ovaries was checked by observation through the membrane between the cephalothorax and the abdomen. Females whose ovaries were small and pale yellow (undeveloped) were included in the experiment whereas those with richer yellow or large red ovaries (fully developed) were not.

After either 6 or 14 days acclimation and 48 h starvation, each individual was given 350 mg (DW) of either the decayed or undecayed Elodea which had been rehydrated for two hours under sterile conditions. Crayfish were left to feed for 12 hours. At the end of this period uneaten food was removed and dried to constant weight. Crayfish were placed in containers without food and faeces were collected during the following 48 hours, concentrated on filters (Whatman GF/C on Millipore apparatus) and dried. Organic carbon content of all food and faecal samples was measured by dichromate oxidation (Winterbourn, Hildrew & Box, 1985; Collier, 1987) and energy equivalents (joules) were calculated from carbon values as described by Newell (1982). Oxidative efficiency was checked by comparison with energy values from bombed food samples (Gallenkamp Ballistic Bomb Calorimeter) and corrected as appropriate. Ingestion (I) was calculated as food provided (mg DW) - food remaining (mg DW) and assimilation efficiency (%) as, I (joules) - Egestion (E, joules) / I (%).

Dichromate oxidation was used in preference to ash-indicator methods (Conover 1967) because neither food calcium concentration nor state of calcium metabolism of the crayfish were known. Lack of information on either may have resulted in errors in estimation of assimilation efficiency. Oxidation also gives a more direct measure of energy assimilated.

Data were analysed using nested ANOVA on a Burroughs computer.

4.2.2 Effect of temperature on enzyme activity

Eighteen crayfish (9 males, 9 females) of similar size (10-12 g) were selected at random from a collection of animals made 2 weeks previously. They were isolated in individual opaque cylindrical, flat - bottomed plastic containers (160 mm diam. * 110 mm deep). The containers had 1 mm mesh lids and mesh - covered holes in the sides (6 per container ; 25 mm diam) to allow water to circulate. Sets of three containers containing animals of one sex were placed in large plastic tanks (172 mm deep * 470 mm * 265 mm) filled with tap water at ambient temperature (about 17^{o}C) and fitted with an aerating activated-charcoal filter. Two tanks (one containing males, the other females) were placed in each of three temperature - controlled rooms at 5^{o}C, 15^{o}C and 20^{o}C (12/12 LD cycle) for two weeks. During this period the animals were fed a mixture of mud (including detritus) from Lake Georgina and Elodea which had been allowed to decay for a month in aerated lake water at 15^{o}C. To reduce stress caused by people moving in the rooms, each tank was surrounded by a cardboard screen which

also served to reduce light levels during the day. Charcoal used in the filters was replaced after 5, 9 and 13 days and food was replenished every three days.

On the fifteenth day all animals were killed by freezing (-80°C) and their hepatopancreases were excised. Each hepatopancreas was homogenised in 3 ml sterile distilled water. After centrifugation (10000 g, 4°C, 20 min) the supernatant (extract) was removed. Three sub-samples of 0.5 ml taken from each hepatopancreas extract were incubated with 15 mg of freeze - dried, powdered Elodea for 18 h at 15°C. At the end of the incubation, samples were filtered (Whatman GF/C on Millipore apparatus) to remove substrate particles and total reducing sugars were measured as described in Section 3.2.. Replicates containing aliquots of boiled extract served as controls throughout.

The entire experiment was repeated subsequently using amylose (2% w/v) rather than powdered Elodea as substratum, and data were analysed as described in Section 4.2.1.

4.3 Results

4.3.1. Feeding rates and Assimilation

Feeding rates of large crayfish (38-45 g) offered decaying Elodea in laboratory trials at 15°C after 6 days acclimation were significantly lower than those of small crayfish (10-12 g) under

the same conditions (**Table 4.1**). Mean assimilation efficiency of large crayfish was also lower, but because individual variation was high no significant differences were apparent (nested ANOVA, $P > 0.05$).

Feeding rates of small crayfish offered Elodea were highly variable and ranged from 3 to 73 mg DW /g DW /d (**Table 4.1**) depending on experimental conditions and food. No significant differences were found between the sexes or when fresh or decaying Elodea was offerred as food (nested ANOVA, $P > 0.05$). Gut evacuation was about threequarters complete within 24 hours (78 ± 26 %; $n = 20$) and was complete within 48 hours. At $5^{o}C$, mean ingestion rate was only 1 mg DW /g DW /d, but assimilation efficiency was very high (87 %). In contrast, mean assimilation efficiency in five trials at $15^{o}C$ ranged from 12 to 34 %, but because individual variation was again high no significant differences between treatments or sexes were apparent (nested ANOVA, $P > 0.05$).

4.3.2 Effect of temperature on enzyme activity

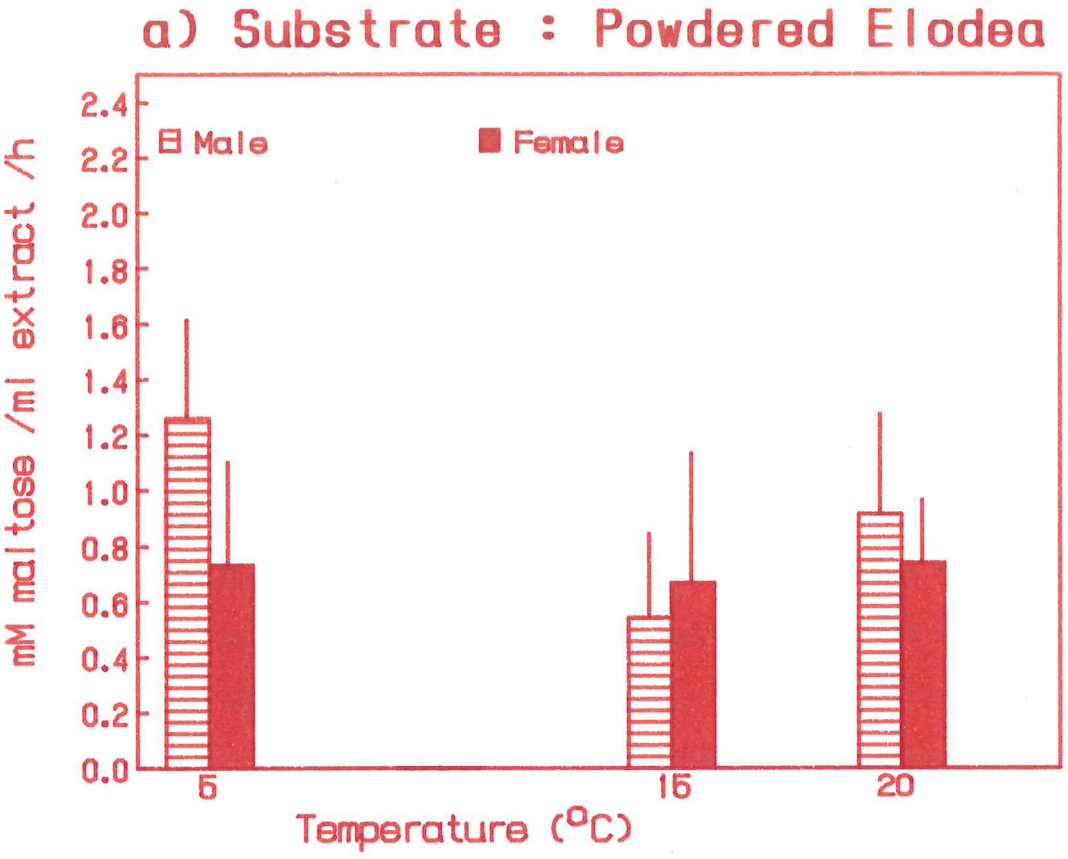
Assays with Elodea as substrate showed greater activity than those using amylose, presumably because increased substrate complexity allowed involvement of a greater range of enzymes.

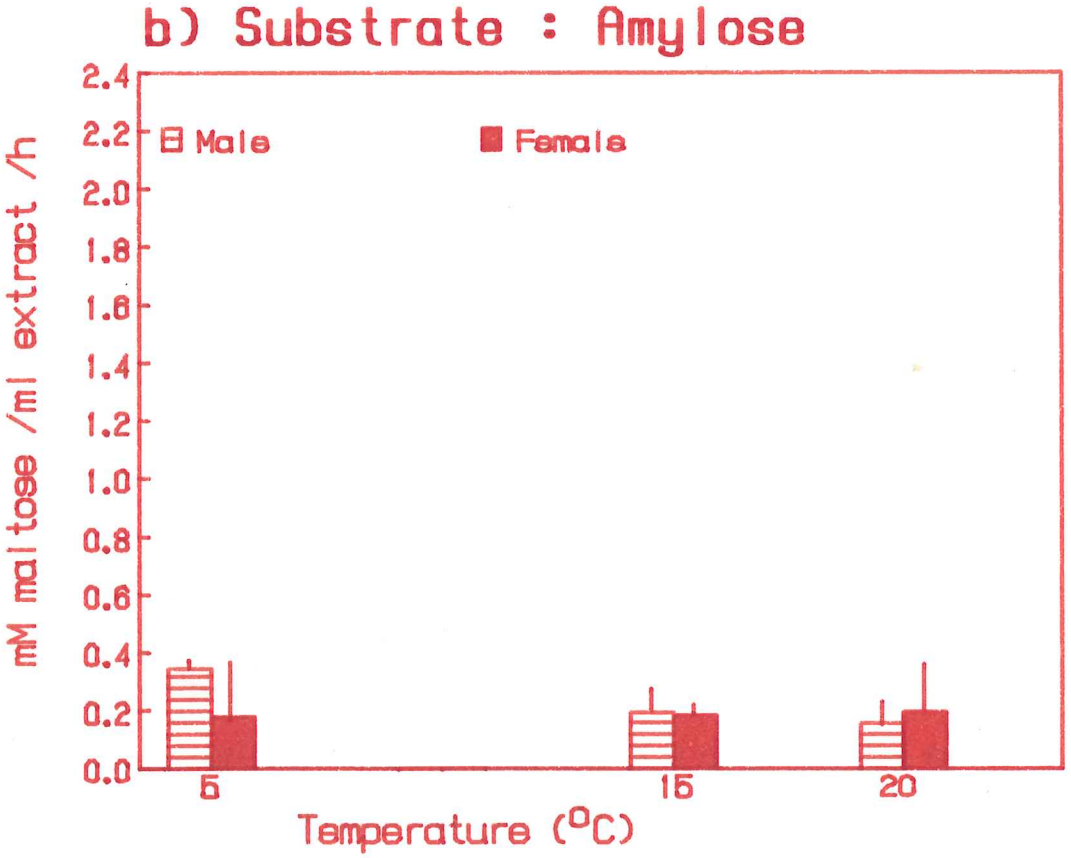
Males acclimated at $5^{o}C$ and tested at $15^{o}C$ showed significant increases (nested ANOVA, $P < 0.001$, **Fig 4.1**) over controls acclimated and tested at $15^{o}C$. In both experiments, mean enzyme activity of males acclimated at $20^{o}C$ did not differ significantly

TABLE 4.1 Ingestion rate and assimilation efficiency (mean and range) of P. zealandicus fed fresh and decaying Elodea canadensis in 12 hour laboratory experiments. N - number of crayfish. Probability level for significantly different results (Students t) * = 0.05.

Food Condition	Crayfish Fresh Weight (g)	Temperature (°C)	N	Acclimation time(days)	Ing (mg/g/day)	AE (%)
Fresh	10 - 12	15	10	6	46.8 (19.2 - 73.2)	15 (4 - 47)
	"	5	6	14	1.0* (0.5 - 1.2)	87* (79 - 94)
Decayed	"	15	6	14	3.7 (3.0 - 4.2)	34 (8 - 58)
4 weeks	"	15	10	6	14.8 (11.8 - 14.9)	28 (13 - 64)
	38 - 45	15	10	6	4.0* (2.8 - 4.9)	17 (10 - 27)
Decayed 6 weeks	10 - 12	15	10	6	40.5 (9.2 - 64:8)	13 (2 - 45)

Fig 4.1 The effect of temperature on carbohydrase activity. Each point represents the mean of nine replicates. Vertical bars = 95% confidence limits.


a) Substrate : Powdered Elodea
mM maltose /ml extract /h
2.4
2.2
2.0
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
Male
Female
5
15
20
Temperature (°C)


b) Substrate : Amylose
mM maltose /ml extract /h
2.4
2.2
2.0
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
Male
Female
5
15
20
Temperature (°C)

from that of males at 15°C but was lower than that recorded from animals kept at 5°C when amylose was used as substrate. Enzyme activity of female crayfish was highly variable and no temperature effects were discernible.

4.4 Discussion

The state of decay of Elodea leaves had no apparent effect on ingestion rate or assimilation efficiency (measured in energy units) in laboratory experiments, but it is probable that the actual material digested differed depending on the state of leaf decay. Thus physical breakdown of living plant material by the gastric mill gives enzymes access to cell contents, whereas microbial biomass (fungi and bacteria) and algae are likely to be significant, digestible components of detritus. Assimilation efficiencies of P. zealandicus fed detritus were highly variable but highest at low temperatures whereas ingestion rates, although also variable, were lowest at low temperatures.

Smaller crayfish ingested more food per gram body weight than larger individuals. Mean assimilation efficiency was also higher in small crayfish but individual variation masked possible significant differences. Data from lake (Devcich, 1979) and stream (Hopkins, 1967) populations of P. planifrons, the other New Zealand freshwater crayfish suggest that young adult P. zealandicus from Lake Georgina (10-12 g crayfish) may moult 2 or 3 times a year and older individuals once or twice. Increased metabolic costs associated with a higher moult frequency may be a

reason for the greater specific ingestion rate of smaller individuals.

The hypothesis that lower temperatures result in greater enzyme activity was supported by the experimental results obtained with males. Why females showed no comparable response is unclear. In both experiments, females showed greater variability and lower mean enzyme activity at $5^{o}C$ than males and this may reflect an interaction between a metabolism geared to ovarian development and 'unseasonally' low experimental temperatures.

The higher assimilation efficiency at low temperatures may compensate to some extent for reduced ingestion, and it is possible that it is a consequence of enhanced enzyme activity. Thus, slower gut passage time and/or reduced gut loading may give enzymes greater access to food and consequently allow more complete digestion.

CHAPTER 5

SUMMARY

SUMMARY

The following is a summary and synthesis of the findings discussed in the previous chapters.

The diet of the crayfish in Lake Georgina during the period of the study was comprised largely of macrophyte detritus (Elodea canadensis), epilithic algae and exoskeletal material. The latter two components appeared periodically, their incidence being related to periods of enhanced epilithic algal production and crayfish growth. Fish and other animal tissue appeared rarely in guts, probably reflecting the faunal paucity of the lake. Fish was the most significant non-exoskeletal animal tissue in terms of both points and abundance. No differences were found in diet between sexes or sizes of crayfish.

General activity of crayfish was higher in May than January, which could indicate an autumnal period of energy accumulation for use in winter when activity was lowest. Activity was again high in September when there was an increase in moult frequency and egg production.

Digestive enzyme activity was investigated in a series of laboratory assays. Hepatopancreas extracts containing digestive enzymes showed catalytic activity toward microcrystalline cellulose (MCC), carboxymethyl cellulose (CMC), cellobiose, amylose, pectin, mannan, laminarin, chitin and 'Azocoll'. Microbial activity was implicated in the breakdown of MCC, laminarin and protein. Endogenous enzyme activity was not detected

in the assays with MCC suggesting a solely microbial source for this enzyme. Although the identity of the contributing micro-organisms is unclear, three genera of Enterobacteriacae were isolated from digestive juices and hepatopancreas samples. It is suggested that the gut and hepatopancreatic environments are amenable to microbial life and that the latter organ may act as a collection site.

Although cellulose cannot be broken down without some degree of microbial conditioning, the polytrophic feeding strategy of P. zealandicus is indicated by the presence of autogenic enzymes that hydrolyse storage and structural sugars of algae, fungi and higher plants as well as animal protein.

The effect of temperature on enzyme activity, and the effect of temperature, size, sex and food condition on ingestion rate, and assimilation efficiency were investigated.

Rates of ingestion of fresh and decaying Elodea canadensis were highly variable at 15^{o}C and assimilation efficiency averaged 21 %. At 5^{o}C, feeding rate was greatly reduced but mean assimilation efficiency was 87 %. Large crayfish ingested significantly less Elodea than small crayfish under the same conditions without differences in assimilation efficiencies. The significance of this result is discussed in the light of the differing energetic requirements of non-breeding large and small crayfish. Differences in sex and food condition did not affect assimilation efficiency or ingestion.

Male crayfish showed increases in enzyme activity at low temperatures when amylose and powdered Elodea were provided as substrates, but females showed little response to temperature.

This may have been related to sex-specific stress during the experiment. It was concluded that the higher assimilation efficiency at low temperature may compensate for reduced ingestion to some extent, and it is possibly a consequence of enhanced enzyme activity. Thus, slower gut passage time and/or reduced gut loading may give enzymes greater access to food and consequently allow more complete digestion. However, the need for energy accumulation prior to winter, suggested by increased activity in May implies that the degree of compensation may not be adequate to maintain metabolic requirements during the coldest months.

In summary, temperature appears to be a major abiotic influence on the crayfish population of Lake Georgina. It influenced epilithic algal production and crayfish distribution and both physical (i.e. ingestion and activity) and metabolic (i.e. enzymic catalysis and assimilation efficiency) activity. Photoperiod also had an important effect, interacting with temperature to maximise summer epilithic algal production and influence diel crayfish activity.

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